SYNTHESIS AND INTERACTION OF SOME OLIGOPEPTIDE AMIDES AND BIS-REPORTER MOLECULES WITH SALMON SPERM DNA

By

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TO MY FAMILY

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The author will always remember Professor E.J. Gabbay for his scientific guidance, friendship and encouragement. He was a dear friend.

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Abstract of Dissertation Presented to the Graduate Council
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SYNTHESIS AND INTERACTION OF SOME OLIGOPEPTIDE AMIDES AND BIS-REPORTER MOLECULES WITH SALMON SPERM DNA

By

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March 1981

Chairman: Edmond J. Gabbay (deceased), Merle A. Battiste
Major Department: Chemistry

The synthesis and interaction specificities of eleven oligopeptide amides with Salmon Sperm DNA forms the principal basis of this dissertation. For all oligopeptide amides the N-terminal amino acid residue was an L-lysyl residue. Dipeptide amides had a dinitrophenyl (DNP, reporter) group attached from either the α- or ε-amino group of a lysyl residue. Amino acid residues at positions two and four of the peptide chain for the tetrapeptides and at positions two and six of the peptide chain for hexapeptides contained an aromatic moiety. Either one or three amino acid residues were used as spacers in between the aromatic amino acid residues.

Oligopeptide amides 1-6 contained tyrosyl and phenylalanyl residues in which the sequence and chirality of the phenyl residue were varied. The rationale for this series was to determine if bis-partial intercalation of the aromatic residues in between base pairs of DNA was observed as a function of the spacing in between aromatic residues.

Oligopeptide amides 7-11 contained dinitrophenyl (DNP) derivatives of lysine. In dipeptides 7-9 the positions of the DNP were varied in order to determine the sequence dependence on intercalation of the reporter group with DNA. Bis-reporter oligopeptide amides 10 and 11 contained two ε-DNP-L-lysyl residues. The rationale for this series was to incorporate bis-intercalators in molecules having a rigid backbone as a function of the spacing in between aromatic residues.

The extent and the mode of interaction of these oligopeptide amides with Salmon Sperm DNA were determined by a variety of techniques: 1) ^{1}H NMR studies, 2) viscometric titrations, and 3) u.v. absorption and CD studies.

It was found that data consistent with partial intercalation were obtained only with phenylalanyl residues. This interaction was found to be sequence dependent and also dependent on the chirality of the phenylalanyl residue. An apparent difference in the interaction of phenylalanyl and tyrosyl residues with DNA was observed.

Mono-reporter molecules were found to intercalate their aromatic moieties in between base pairs of DNA. Bis-reporter molecules gave data suggestive of bis-intercalation.

CHAPTER 1

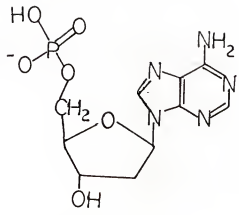
INTRODUCTION

The interactions of small molecules with nucleic acids have been studied extensively by a variety of physical techniques.[1] These have yielded considerable information about the kinds of molecules that bind to nucleic acids and of the types of interactions present. These small molecules include mutagens and carcinogens (such as aminoacridines[2] and polycyclic aromatic hydrocarbons),[3] antibiotics (such as actinomycin D),[4] a variety of planar dyes (such as Ethidium)[5] and numerous heavy metals[6,7] (some of which bind to phosphates and some to the bases).

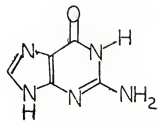
In addition to small molecules, macromolecules such as proteins also bind to nucleic acids. Proteins are responsible for packaging DNA into chromatin,[8] are involved in the replication of DNA,[9] and in the regulation of nucleic acid function.[10]

Structure of DNA

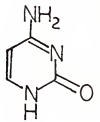
Knowledge of the general structure and properties of nucleic acids is essential in order to understand their interactions with other molecules. The repeating unit in deoxyribonucleic acid (DNA) is the nucleotide. Nucleotides are composed of a heterocyclic base (purine or pyrimidine) and a deoxyribose sugar which is esterified at the 5' position by phosphoric acid. The bases, which consist of two purines, adenine (A) and guanine (G), and two pyrimidines, thymine (T), and cytosine (C), are connected through β-glucosidic linkages to the C-1' carbon of the deoxyribose sugar (fig 1-1).



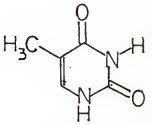
dAMP



G



C



T

Fig 1-1. Structure of a nucleotide (d AMP) and of purine and pyrimidine bases, Adenine (A), Guanine (G), Cytosine (C) and Thymine (T).

A single strand of DNA consists of nucleotides joined together through phosphodiester bonds at both the C-3' and C-5' hydroxyl groups of the deoxyribose sugar (fig 1-2). A single stranded polynucleotide can be of variable length and will always have a free C-3' hydroxyl group at one end of the strand and a C-5' hydroxyl group at the other end of the strand.

It has been generally assumed that DNA in aqueous solution exists primarily in the B conformation,[11,12] which was originally proposed by Watson and Crick in 1953.[13,14] The B conformation of DNA is one of the several double helical geometries of DNA defined by fiber x-ray diffraction and it is found in sodium-ion containing fibers at high relative humidity (fig 1-3). It consists of two polynucleotide strands wound around each other into a right handed double helix with a cylindrical diameter of about 20 Å. The bases are within the hydrophobic interior of the double helix, while the hydrophilic sugar residues and phosphate anions are located on the periphery of the double helix.

The two polynucleotide strands are antiparallel and run in opposite directions with the 3' terminus of one chain and the 5' terminus of the opposite chain at the same end of the double helix. The bases are perpendicular to the helix axis and arranged in such a way that adenine is always hydrogen bonded to thymine and guanine is always hydrogen bonded to cytosine. The A:T base pair is held together by two hydrogen bonds and the G:C base pair by three hydrogen bonds (fig 1-4). The hydrogen bonded base pair forms a plane that is 3.4 Å in thickness, which is just the van der Waals radius of an aromatic ring.

The sugar phosphate chains are separated from one another by 120° and this results in the formation of a major and a minor groove in the double helix. These grooves can be seen as depressions between the negatively charged, hydrophilic sugar phosphate backbones. The grooves are

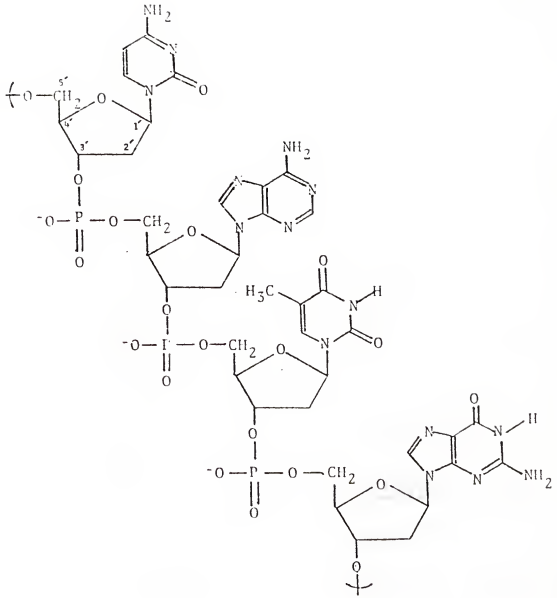


Fig 1-2. Structure of a section of a DNA chain.

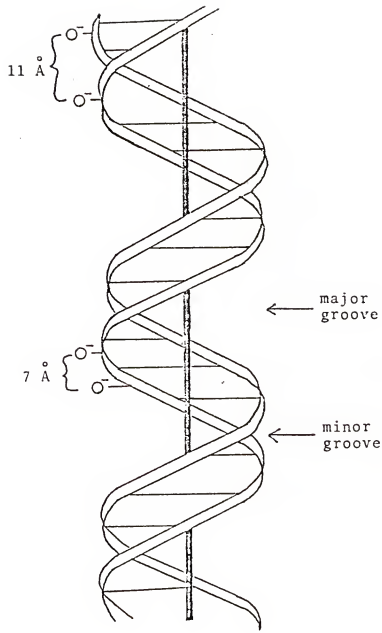
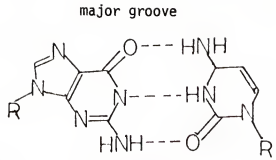
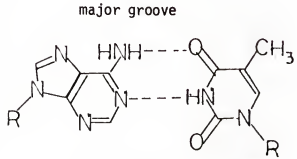


Fig 1-3. Schematic representation of the Watson-Crick double helix of DNA. The outer helical strands represent the sugar-phosphate backbone, while the horizontal lines represent the base pairs and the vertical line is the helical axis.



G-C



A-T

Fig 1-4. Watson-Crick base pairs.

unequal in size, the major groove being about 22 Å across, measured between phosphates in the direction of the helix axis, while the minor groove is about 12 Å across on the same basis. The grooves are both about 7 Å in depth, measured from the surface of the enveloping cylinder.[15]

X-ray work with DNA requires the use of fibers. Slightly different structures can be obtained by varying the relative humidity of the fibers or the counterions.[15,16] The known helical structures of double stranded nucleic acids differ in a number of important features. These include the number of base pairs per turn of the helix, the tilt of the base pairs (the angle between the base pair and the helix axis), the twist of the base pairs (the angle between planes containing two paired bases), the diameter of the helix, and the nature of the grooves of the helix. In the A form, the base pairs are tilted about 20° from the helix axis and there are 11 base pairs per turn with a rise per residue of 2.82 Å.[15] The C form resembles the B, but has only 9.3 base pairs per turn and a rise per residue of 3.32 Å.

Recently, a left handed double helical structure has been found for a synthetic deoxyribose hexanucleotide.[17] It has the alternating sequence $d(CpG)_3$ and it was named Z DNA since the phosphate groups seem to pursue a zigzag course along the backbone.

In cells and viruses, the DNA is found packaged into fairly compact structures. Therefore, it is of great interest to know more about the DNA tertiary structure, especially how the rigid DNA double helix can be bent or folded.

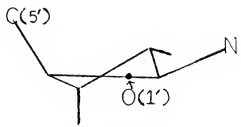
Several researchers have speculated on some possible aspects of DNA folding. Kinked DNA was first proposed by Francis Crick and Aaron Klug[18] and it can form in the DNA helix by unstacking two adjacent base pairs

and untwisting the DNA backbone a bit. The stereochemistry of possible kinks has been studied extensively by Sobell et al.[19] through molecular model building. Kinks can occur only in one direction relative to the backbone and if kinks are placed into the helix at regular intervals, superhelical DNA structures can be generated. Kinks can be generated by altering the sugar pucker at the place of bending from all C-2' endo in B-DNA to C-3' endo (3'-5') C-2' endo across the kink (fig 1-5). It has been suggested that this change in sugar pucker may also occur upon intercalation of a planar aromatic molecule in between base pairs of DNA.

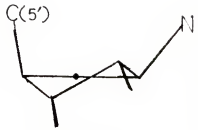
Other researchers view DNA as a flexible macromolecule. Bending of this flexible DNA molecule can be achieved by small perturbations in the rotation angle of the polynucleotide backbone. Wilma Olson[20,21] has shown that a wide variety of DNA superhelices can be generated by regularly bending a B-DNA reference helix.

The stability of the helix depends on a delicate balance between unfavorable interstrand phosphate anion repulsions and favorable base pairing and stacking. Base pairing interactions have generally been studied in nonhydroxylic solvents.[22] In aqueous media, base pairing seems apparently unimportant due to possible hydrogen bonding interactions between the bases and water. However, the interior of a DNA double helix is very nonpolar and the exclusion of water from this region due to the hydrophobic nature of the bases provides an ideal environment for base pairing and significant stability to the double helix.

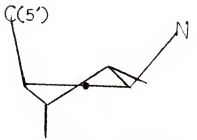
Base stacking interactions occur in aqueous solution and have been observed in single stranded polynucleotides,such as poly A,[23] and between free monomeric nucleosides. The interaction is associated with a negative ΔH, unlike pure hydrophobic interactions which are endothermic and,



C(2')-endo



C(3')-exo



C(2')-endo-C(3')-exo

C(3')-endo

Fig 1-5. Representations of several conformations of a ribose or deoxyribose ring in a nucleoside.

consequently, entropically driven. Some data suggest that hydrophobic effects play a significant role in stabilizing base stacks[24] but it cannot be unequivocally said that hydrophobic interactions along lines of those for apolar molecule interactions in water are the primary source of stabilization.

Small Molecule-DNA Interactions

Small molecules may bind to nucleic acids via electrostatic, hydrogen bonding and hydrophobic interactions. The phosphate groups make DNA a polyanion and give the molecule a strong affinity for cations, as exemplified by the strong binding of lysine rich proteins to DNA.[25] Hydrogen bonding to functional groups of the purine and pyrimidine rings not involved in base pairing is possible. Hydrophobic interactions are of particular interest and two types have been noted 1) intercalation (full or partial) and 2) external hydrophobic binding.[26]

Classical Intercalation

Intercalation or insertion of planar aromatic cations between base pairs of DNA has been studied extensively and was first proposed by Lerman.[27,28] Intercalation involves an unwinding of the deoxyribose sugar phosphate chain in order to create suitable spaces, around 3.4 Å in thickness, to accommodate the incoming aromatic cation. The intercalation process brings about detectable characteristic changes in the hydrodynamic properties of DNA as well as changes in the physical and chemical properties of the intercalated molecule.[29]

Evidence in favor of an intercalative mechanism has been obtained from studying the hydrodynamic properties of DNA and detecting changes of these in the presence of the ligand.

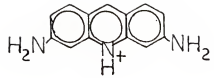
The viscosity of a DNA solution is markedly increased, while the sedimentation coefficient decreases upon binding of an intercalator such

as acridine orange, proflavine or ethidium (fig 1-6). The increase in the intrinsic viscosity of the DNA upon binding an intercalator can be related to the length increase caused by the intercalator on DNA, whose shape can be considered similar to that of a rigid rod for short double helical segments (sonicated DNA).[30,31] The decrease in the sedimentation coefficient is associated with a decreased mass per unit length.

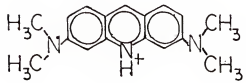
More recently, the use of closed circular superhelical DNA in the characterization of the intercalation process has shown that unwinding of the helix occurs upon intercalation and has allowed determination of intercalation unwinding angles.[5,32-34] The intercalation unwinding angle is the amount by which the usual rotation between base pairs (36°) is reduced when neighboring base pairs are separated to create space for intercalation of the ligand.

The interaction of an intercalator with closed circular superhelical DNA can be monitored by viscometry and sedimentation methods. The unwinding that results upon intercalation initially removes the right handed superhelical turns (coils) until an uncoiled circular DNA is obtained, addition of more ligand will cause continued unwinding and supercoiling in the reverse direction. The uncoiled form of the closed circular DNA is the most extended and therefore, exhibits the highest intrinsic viscosity and the lowest sedimentation coefficient.

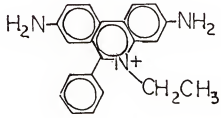
Definitive proof for intercalation is available from x-ray diffraction studies of several intercalators with DNA as well as with ribose and deoxyribose dinucleotides. Fiber diffraction studies of several intercalator-DNA complexes[29,35] show a retention of its 3.4 Å spacing with the base pairs essentially perpendicular to the helix and loss of the regular repeating nature of the helix. Recently, the x-ray fiber diffraction of a platinum containing intercalator reagent[36] (fig 1-6) with DNA complex



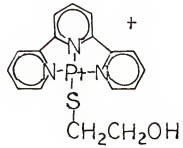
Proflavine



Acridine Orange



Ethidium



2-Hydroxy ethane-thiolato terpyridine platinum II

Fig 1-6. Structures of some common intercalators.

showed the standard 3.4 Å reflection and an additional meridional line at a position corresponding to a spacing along the helix of 10.2 Å. This is the expected repeat distance of binding one intercalator molecule every other base pair (at saturation of intercalation sites) according to the nearest neighbor exclusion principle.[37]

Dinucleotides-intercalator x-ray studies[38-42] are particularly informative and have yielded information about the sugar puckering at the intercalation site, changes in backbone torsional angles, unwinding angles and positioning of the base pairs with respect to each other and with respect to the intercalated molecule. However, it is difficult to extrapolate the results from dinucleotides to high molecular weight natural DNA.

Upon intercalation, changes in the physical and chemical properties of the intercalator's aromatic moiety are observed. The visible electronic spectrum of the DNA bound molecule is found to shift to longer wavelengths and to exhibit considerable hypochromism.[43] The fluorescence emisson quantum yield of the DNA bound molecule can either be reduced (quenched) as with proflavine[44] or enhanced as with ethidium bromide.[45] Induced circular dichroism at the electronic transition of the intercalated molecule is observed upon binding to DNA.[46] ^{1}H NMR studies with DNA-intercalator complexes show total line broadening of the aromatic protons of the ligand.[47,48]

Non-classical Intercalation

Partial intercalation has been proposed as the mode of binding of some sterically restricted aromatic molecules to DNA.[49,50] This mode of binding has been characterized by an apparent shortening of the helix as seen by a decrease in the viscosity of DNA upon binding of the ligand. It has been proposed that a bending of the helix occurs at the point of insertion.

Evidence in favor of a partial intercalation mechanism has been obtained by Kapicak and Gabbay[49] by studying model systems of the type shown in fig (1-7).

These studies revealed that the extent of insertion of the aromatic ring between base pairs of DNA had a profound effect on the tertiary structure of the helix. This type of molecule can interact with DNA in two ways, by electrostatic interactions of its positively charged ammonium groups and adjacent negatively charged phosphate groups on the DNA sugar phosphate backbone, and by stacking interactions of its nitrophenyl ring with the base pair of DNA. For n=1, the nitrophenyl ring was found to be partially inserted between base pairs of DNA as evidenced by a lower viscosity of the DNA complex and by lack of ^1H NMR signal broadening of the aromatic protons. A greater upfield chemical shift was found by the A protons than for the B protons in the DNA complex, which is consistent with a partial insertion model. At n=2,3 and 4 the nitrophenyl ring can fully insert between base pairs and causes a net increase in the helix length.

Additional evidence for bending of the helix was reported by Gabbay and coworkers[50] by studying a series of symmetrically and asymmetrically methylated phenanthrolines (fig 1-8).

It was found that the extent of substitution and the position of the substituents affected the observed viscosity increase with respect to the unsubstituted compound. Symmetrical and highly substituted derivatives increased the viscosity of DNA solutions much more than the unsubstituted compound. This was attributed to the increased effective thickness of the ring system when extensively methylated. The asymmetrically substituted derivatives gave the smallest viscosity increase of all the derivatives studied, even smaller than the unsubstituted compound.

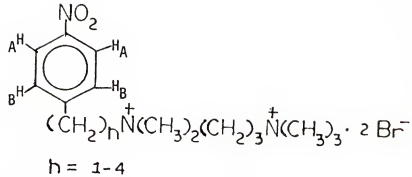


Fig 1-7. Structure of p-nitrophenyl dications.

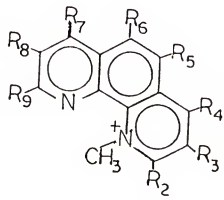


Fig 1-8. Structure of N-methyl phenanthrolinium cations.

a) symmetrically $R_2 = R_5 = R_6 = R_9 = CH_3$ methylated

b) unsymmetrically $R_2 = R_9 = CH_3$ methylated

The asymmetrically substituted derivatives bind by intercalation but can cause both lengthening and bending of the helix (fig 1-9). The net result is a smaller increase in the effective hydrodynamic helix length and viscosity than is produced by a classical intercalative mechanism.[50]

Partial intercalation has also been proposed as a possible mode of binding of aromatic amino acid residues to DNA. Brown[51] was the first to suggest a "selective bookmark" hypothesis whereby the aromatic amino acid residues may serve as anchor and prevent slippage of the protein along the DNA.

Gabbay and coworkers have studied extensively the interactions of peptides containing phenylalanine residues with nucleic acids.[52-56] These peptides stabilize DNA against thermal denaturation and decrease the specific viscosity of DNA to a greater extent than similar peptides containing no aromatic side chains. Furthermore, for some of these peptides the ^{1}H NMR of the aromatic region exhibit upfield chemical shifts as well as broadening of their signals.

Results from the studies of a large number of phenylalanine containing peptides showed that whether data consistent with partial intercalation of the aromatic residue were obtained depended on 1) its position in the sequence, and 2) on its stereochemistry. This is illustrated by Gabbay's study of the interaction of L-lys-L-pheA and L-lys-D-pheA with Salmon Sperm DNA.[55] These diastereomers show a stereospecific interaction with DNA. The dipeptide L-lys-L-pheA exhibits a larger decrease in the relative specific viscosity of the DNA solution than the corresponding diastereomer L-lys-D-pheA. The ^{1}H NMR signal of L-lys-L-pheA in the presence of DNA exhibits two broad resonance lines which are shifted upfield with respect to the free peptide by 6.0 and 23.5 Hz, while for its diastereomer slight broadening and upfield chemical shifts are observed.

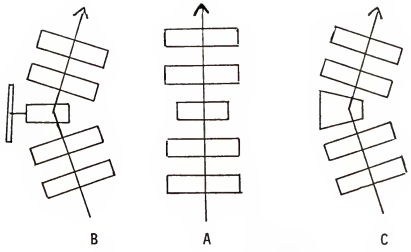


Fig (1-9)a

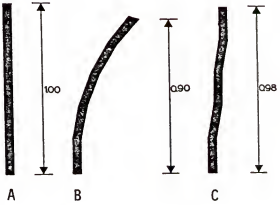


Fig (1-9)b

a. Classical and nonclassical intercalation models
   A - classical intercalation
   B - nonclassical intercalation involving sterically restricted aromatic molecules
   C - nonclassical intercalation involving an asymmetrically substituted chromophore

b. Schematic representations illustrating the decrease in helical length due to partial intercalation of an aromatic molecule
   A - structure represents unit length of helix
   B - helix with consecutive 10^{o} helical bends in the same direction
   C - helix with consecutive 10^{o} helical bends in opposite direction

The spin lattice relaxation times T_1[54] are found to be similar in magnitude for the free and bound state suggesting that the phenyl ring of both diastereomers in the DNA complex experience restriction in tumbling of equal magnitude. However, the differences in chemical shifts and line broadening suggest that the phenyl rings of each compound are in different chemical environments in the complex.

A model was proposed in which the terminal L-lysyl residue interacts stereospecifically with the DNA helix (figs 1-10, 1-11) thus dictating the positioning of the aromatic ring of the C-terminal phenylalanine residue.[56] The specificity observed cannot be attributed to the chirality of the phenylalanine moiety itself since ^{1}H NMR studies reveal no significant differences in the binding of L- and D-phenylalanine amides to DNA.[52]

Claude Helene and coworkers have studied extensively the tripeptides LysTrpLys and LysTyrLys with single and double stranded nucleic acids by fluorescence spectroscopy and other techniques.[57-61] The fluorescence of the tryptophyl and the tyrosyl residues are quenched upon binding to nucleic acids. For tryptophyl residues fluorescence quenching was found to be due to stacking interactions between the indole ring of tryptophan and the nucleic acid base pairs, as corroborated by ^{1}H NMR.[57] For tyrosyl residues the fluorescence quenching observed upon binding to nucleic acids has been explained as due to energy transfer to the nucleic acid bases.[60]

Stacking of nucleic acid bases with the aromatic amino acid residues of tyrosine and tryptophan was found to be favored in single stranded nucleic acids as compared to double stranded ones. Helene and coworkers have suggested that stacking interactions of aromatic amino acid residues with nucleic acid bases occur preferentially in unpaired regions of a nucleic acid and that this might be a way of anchoring proteins to nucleic acids.[59]

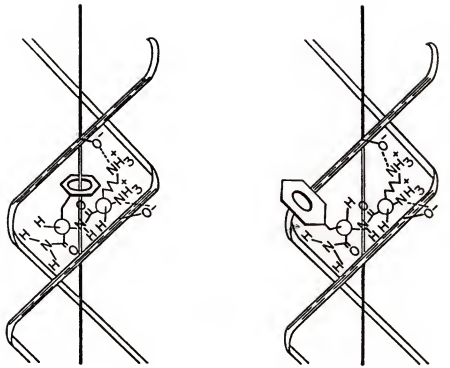
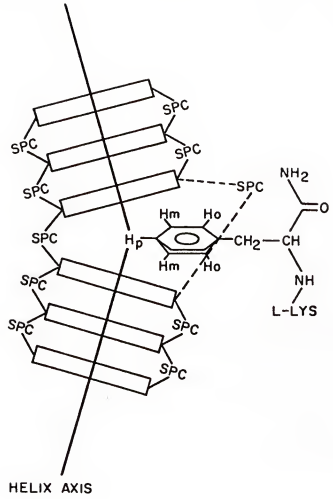


Fig 1-10. Schematic illustration of a DNA segment showing a possible mechanism by which the α- and ε-amino groups of the N-terminal L-lysine residue are anchored stereospecifically and thus dictating the positioning of the C-terminal aromatic ring of phenyl alanine in the complexes with L-lys-L-pheA and L-lys-D-pheA.



DNA–L–LYS–PHEA COMPLEX

Fig 1-11. Schematic illustration of a segment of DNA duplex showing partial insertion of the aromatic ring of L-lys-L-pheA.

Full or partial intercalation of aromatic amino acid residues in between base pairs of DNA is still controversial. Jacobsen and Wang[62] measured the relative sedimentation rates of linear and covalently closed DNA in sucrose gradients containing varying amounts of tryptamine, tyramine, histamine and phenethylamine in order to determine whether aromatic amino acids could intercalate into double stranded DNA. They found that the presence of the aromatic amines did not significantly change the sedimentation coefficient of the closed DNA, indicating that the DNA helix rotation angle was not significantly altered and concluding that intercalation of aromatic amino acid residues into DNA was unlikely.

Quadrifoglio, Giaconti and Crescenzi have proposed a "calorimetric criterion" for intercalation.[63-65] They have found, by studying the interaction of several well-known intercalators with DNA by calorimetry, that the enthalpy of such interaction is large and negative amounting to about minus 5-7 Kcal/mol of drug bound. They also carried out calorimetric measurements of the interaction of small peptides such as LysTrpLys, LysTyrLys, LysTyr, LysTyr amide with DNA and the results of these measurements yielded a zero value for the enthalpy of interaction.[66] They concluded that the tryptophyl and tyrosyl residues of amino acids do not stack or intercalate between base pairs of DNA.

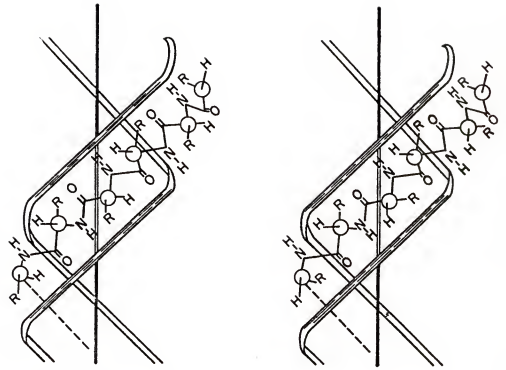
Statement of Problem 1

The interaction of proteins with nucleic acids is a problem of immense complexity since there are probably several types of forces interacting at the numerous sites along the nucleic acid and protein chains.[67] A simplified approach to this problem has been taken by several groups by studying the interactions of amino acids, small peptides and model compounds with nucleic acids in order to provide more insight into the recognition processes between the two macromolecules.[49,56,61]

As was mentioned before, aromatic amino acid residues in proteins could play an important role in the recognition process between nucleic acids and proteins, like anchoring the protein at given sites along the nucleic acid helix. The proposed bending of the helix upon partial intercalation of the aromatic amino acid residues[54] could also be of importance in the folding of nucleic acids by proteins in chromosomes, especially if several of such interactions take place at the same time.

According to Gabbay's earliest studies on the phenylalanine sequence dependence on partial intercalation, an extended β-sheet conformation of these peptides upon binding to DNA (fig 1-12) was proposed whereby the lysyl residue at the N-terminal end of the oligopeptides interacts stereospecifically with DNA.[56] This stereospecific interaction of the lysyl residue seems to allow the phenylalanyl residue next to it to come in closer contact with the nucleic acid bases depending on its chirality.

A series of oligopeptide amides (fig 1-13) containing two aromatic amino acid residues, phenylalanine and tyrosine were synthesized and studied with DNA, in order to determine whether bis-partial intercalation of the aromatic residues could be observed. If the peptides 1, 4 and 5 assume a β-sheet conformation upon binding to DNA, the aromatic residues in positions two and four of tetrapeptides and positions two and six of hexapeptides will be pointing towards the helix and thus should be in close proximity with the nucleic acid bases. In peptides 2, 3 and 6 the extent to which the aromatic residue will point towards or away from the helix, and therefore their degree of interaction with the nucleic acid bases, will depend on the chirality of their phenylalanine residue. If the neighbor exclusion principle holds for small aromatic rings and for the process of partial intercalation, peptides 4 and 5 should be potentially able to bis-partial intercalate whereas peptide 1 should not. For



(a) L-AMINO ACIDS (b) ALTERNATING L,D A ACIDS

Fig 1-12. Schematic illustrations of a model for peptide-DNA binding whereby the polypeptide chain assumes a helical β-sheet structure which is wrapped around the nucleic acid helix. It is noted that for polypeptides composed of L-amino acids, the side chains alternately point into and out of the helix.

1- L-lys-L-tyr-L-ala-L-phe A 2 (OAc^-)

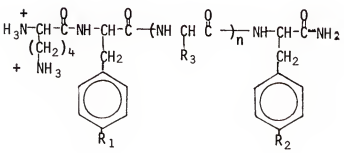
2- L-lys-D-phe-L-ala-L-tyr A 2 (HCO_2^-)

3- L-lys-L-phe-L-ala-D-phe A 2 (OAc^-)

4- L-lys-L-tyr-glyglygly-L-phe A 2 (HCO_2^-)

5- L-lys-L-phe-glyglygly-L-tyr A 2 (HCO_2^-)

6- L-lys-D-phe-glyglygly-L-tyr A 2 (HCO_2^-)



where n = 1 or 3
and when

R_1 or R_2 = -H, phenylalanine (phe)

R_1 or R_2 = -OH, tyrosine (tyr)

R_3 = -H, glycine (gly)

R_3 = $-CH_3$, alanine (ala)

Fig 1-13. Structures of oligopeptide amides 1-6.

peptide 1 the distance between the two aromatic rings is too short to allow for intercalation two base pairs apart upon binding to DNA. Peptides 2 and 6 were synthesized as controls and should give results indicative of mono-partial intercalation upon binding to DNA.

Statement of Problem 2

Bifunctional DNA intercalators are compounds containing two planar chromophore groups each of which is capable of intercalation into double stranded DNA at the same time. It is expected that bis-intercalators will bind more strongly to DNA when compared to the corresponding monomer, and could also be of importance in improving both sequence selectivity and specificity for DNA.

A natural antibiotic, Echinomycin,[68-72] has been identified as a bifunctional intercalator (fig 1-14). It is an extremely potent inhibitor of RNA synthesis and there is every indication that its antitumor activities result from its binding to DNA. Synthetic bifunctional intercalators have been prepared using a variety of chromophores, the most studied being the acridines.[73,74] The two chromophores have been covalently linked together by several types of connecting groups of varying lengths.[75-78]

One of the most important factors affecting the mode of binding of bifunctional intercalators to DNA appears to be the nature, conformation and rigidity of the connecting interchain. Extensive folding of these molecules caused by stacking interactions between the two chromophores has been observed and is believed to limit the solubility as well as the reactivity of bis-intercalators with DNA at physiological pH.[79,80] Recently, evidence has been obtained suggesting that ring substituents on acridine type dimers play an important role upon allowing bis-intercalation to occur.[81]

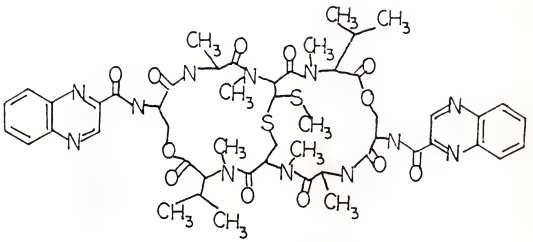
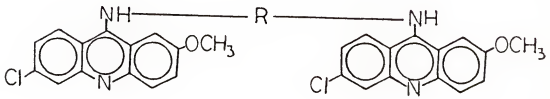


Fig 1-14. Structure of Echinomycin. A natural bis-intercalator.



	R	N*–N* (Å)
1	$[CH_2]_3$ NH $[CH_2]_4$ NH $[CH_2]_3$	16.1
2	$[CH_2]_3$ NH $[CH_2]_4$	11.2
3	$[CH_2]_3$ NH $[CH_2]_3$	9.9

Fig 1-15. Dimers of 2-methoxy-6-chloro-9-amino acridine. N*-N* represents the distance (in Å) between the two nitrogens.

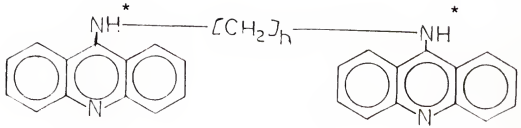
LePecq et al.[73] studied polyamine linked 2-methoxy-6-chloro-9-amino acridines (fig 1-15) by viscometry and fluorescence measurements and concluded that compounds 1 and 2 were able to bis-intercalate but not compound 3 since the interconnecting chain was not long enough to bis-intercalate two base pairs apart (neighbor exclusion principle) which requires a minimal distance of about 10.2 Å.

Wakelin et al.[82] found that for a series of dimeric acridines (fig 1-16) a minimum distance unambiguously permitting bifunctional intercalation was obtained with the n=6 derivative (8.8 Å). These results are consistent only with bis-intercalation occurring on both sides of a base pair which requires a minimum distance of about 6.8 Å. This type of intercalation on both sides of a base pair is in disagreement with the neighbor exclusion principle and had not been observed before.

Wright et al.[81] studied the effect of ring substitution on the bis-intercalation of the acridine dimers. He found that using the same interconnecting chain as LePecq et al.[73] did in compound 3 but using unsubstituted acridine chromophores, results consistent with bis-intercalation were obtained.

As stated before the nature, the conformation and rigidity of the connecting chain of bis-intercalators are believed to be the most important factors affecting the mode of binding of bifunctional intercalators to DNA. So far, interconnecting chains used in bifunctional intercalators have been mainly polyamine chains and aliphatic diamines.

We chose a peptide chain as a logical choice for an interconnecting chain for bifunctional intercalators since it is a rigid chain and it is polar in character. Bis-reporter molecules 10, and 11 (fig 1-18) were synthesized with this concept in mind. Compounds 10 and 11 are bifunctional intercalators where the length of the connecting chain was varied.



n	N*-N* (Å)
2	3.9
3	5.0
4	6.3
5	7.5
6	8.8
7	10.0
8	11.3
10	13.8
12	16.3

Fig 1-16. Structure of Diacridines. N*-N* represents the distance (in Å) between the two nitrogens.

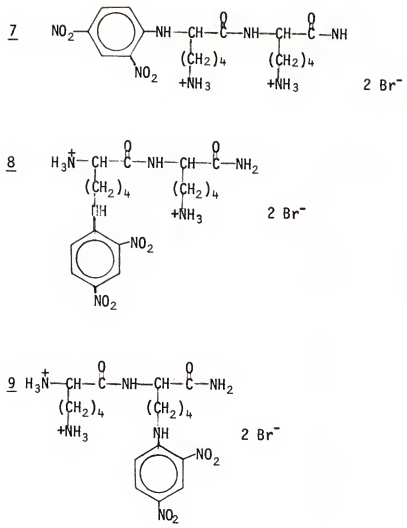
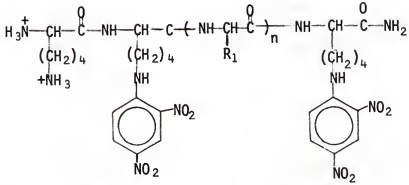


Fig 1-17. Structure of DNP-dipeptide amides 7-9.



10 n = 1; R_1 = $-CH_3$ $2(OAc^-)$

11 n = 3; R_1 = -H $2\ Br^-$

Figure 1-18. Structures of Bis-DNP-peptide amides 10 and 11.

from one to three amino acid spacers in between the amino acid residues containing the chromophores. The length of the interconnecting chain should be long enough to allow bis-intercalation for both cases.[82] Bis-intercalation for compound 11 can occur two or more base pairs apart.

Compounds 7, 8 and 9 (fig 1-17) were synthesized as control and to investigate if there was any sequence dependence for intercalation of the dinitrophenyl moiety. The dinitrophenyl chromophore was chosen for several reasons; it has been extensively studied,[83] bis-intercalators of small ring systems have not been studied and it bears no charge in the ring system.

CHAPTER 2

RESULTS

The interactions of the oligopeptide amides 1-6 (fig 1-13) with Salmon Sperm DNA were studied by ^{1}H NMR, viscometric, and DNA melting temperature (T_m) studies. The interactions of the dinitrophenyl molecules 7-11 (fig 1-17 and 1-18) with Salmon Sperm DNA were studied by viscometric, circular dichroism, DNA melting temperature, spectrophotometric and ^{1}H NMR studies.

^{1}H NMR Studies

The effect of DNA on the ^{1}H NMR spectra of the oligopeptides 1-6 is shown in figures (2-1) - (2-6). Some broadening of all residues of the peptides studied was observed upon binding to sonicated Salmon Sperm DNA at 36°C. For peptides 1, 2, 4 and 6 slight broadening was observed for all residues, aromatic and nonaromatic (alanyl and glycyl). The phenylalanyl residue of oligopeptide 5, L-lys-L-phe-glyglygly-L-tyr A, (fig 2-7) was broadened to a greater extent upon binding to sonicated DNA than any residue in the other peptides with the possible exception of peptide 3, L-lys-L-phe-L-ala-D-phe A.

A small upfield shift (1-3 Hz) was observed for the aromatic residues of most peptides upon binding to sonicated DNA. The observed difference in chemical shifts for the aromatic protons of peptides 1-6 as well as for L-lys-L-tyr A and L-lys-D-phe A in the absence and presence of sonicated and heat denatured DNA are summarized in Table 2-1. Nonaromatic residues (alanyl, glycyl) of all peptides were slightly broadened but were not upfield shifted. Significant upfield shifts were observed only for the phenylalanine residues of peptide 5 (L-lys-L-phe-glyglygly-L-tyr-A)

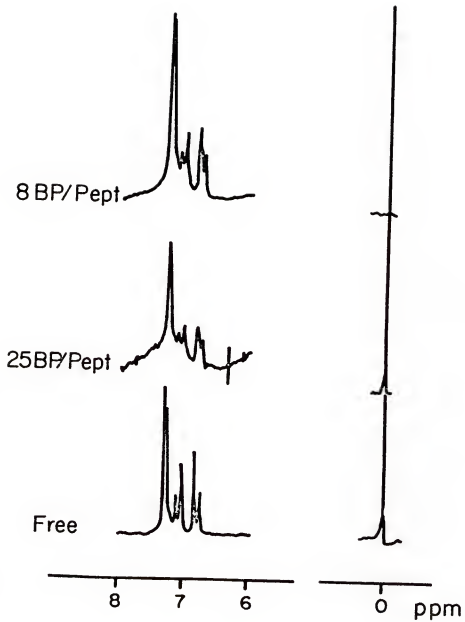


Fig 2-1. ^{1}H-NMR signal of the aromatic protons of L-lys-L-tyr-L-ala-L-phe-A, I (36°C) in the absence and presence of DNA at different DNA base pairs to peptide ratios.

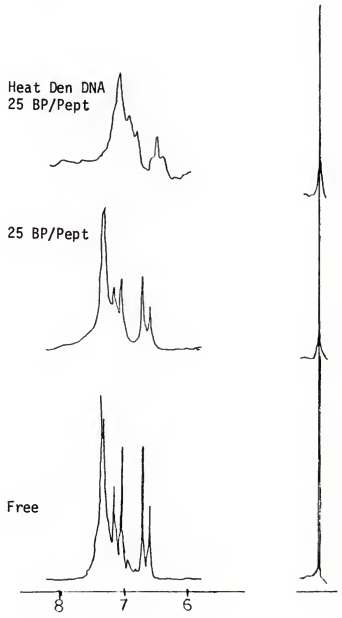


Fig 2-2. ^{1}H NMR signal of the aromatic protons of L-lys-D-phe-L-ala-L-tyr A, 2 (36°C) in the absence and presence of native and heat denatured DNA at 25 DNA base pairs to peptide ratio.

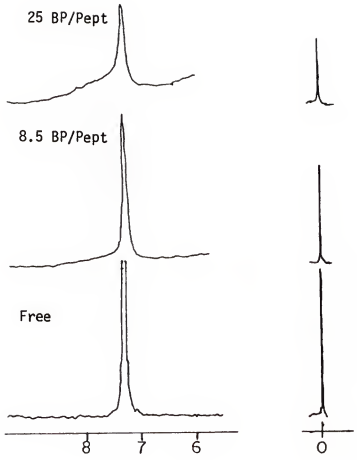


Fig 2-3. ^{1}H NMR signal of the aromatic protons of L-lys-L-phe-L-ala-D-phe A, 3, (36°C) in the absence and presence of DNA at different DNA base pairs to peptide ratios.

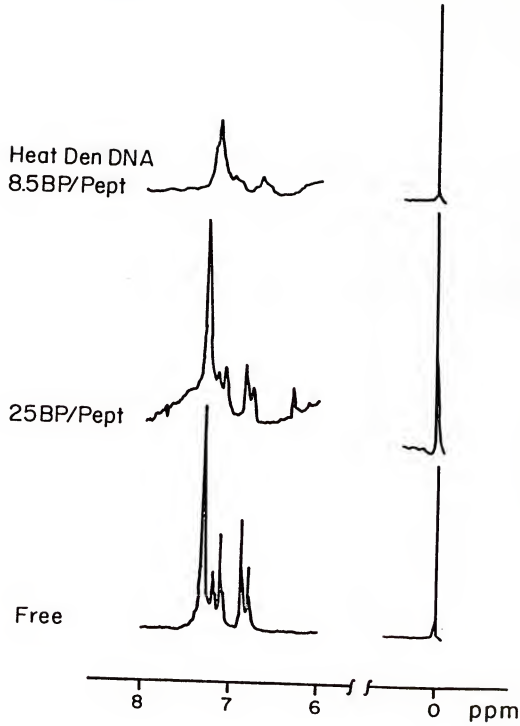


Fig 2-4. ^{1}H NMR signal of the aromatic protons of L-lys-L-tyr-glyglygly-L-phe A, 4 (36°C) in the absence and presence of native and heat denatured DNA at different DNA base pairs to peptide ratios.

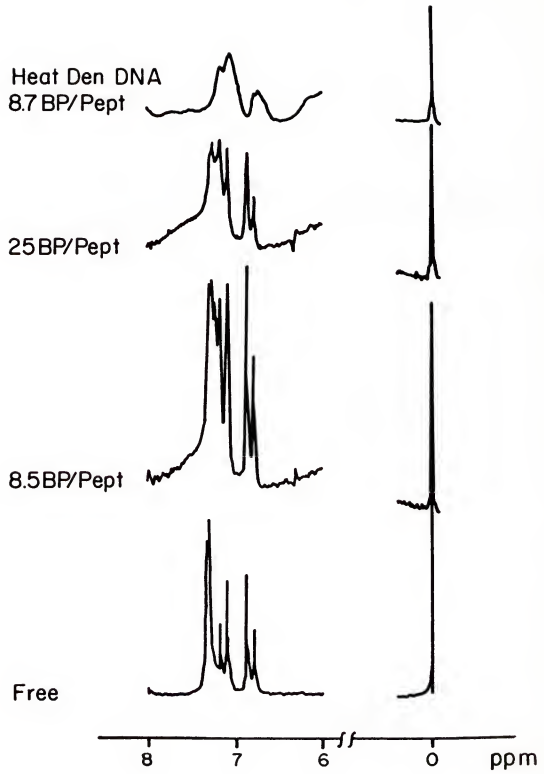


Fig 2-5. ^{1}H NMR signal of the aromatic protons of L-lys-L-phe-glyglygly-L-tyr A, 5 (36°C) in the absence and presence of native and heat denatured DNA at different base pairs to peptide ratios.

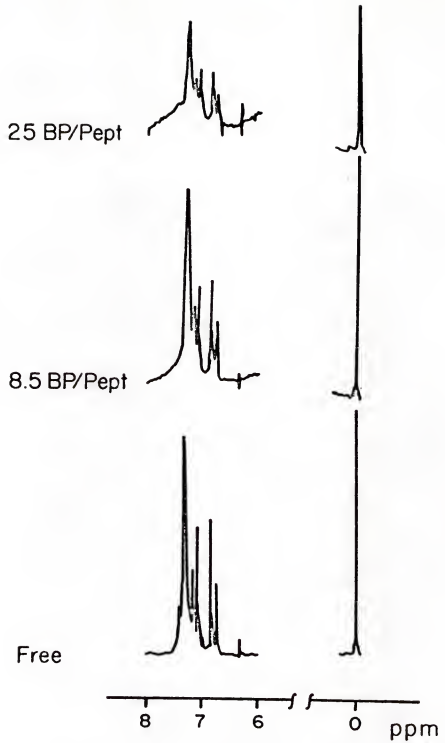
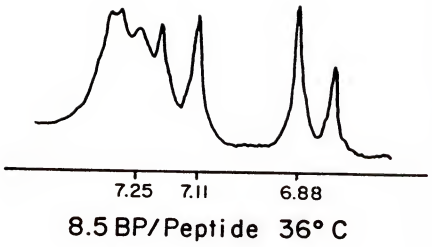


Fig 2-6. ^{1}H NMR signal of the aromatic protons of L-lys-D-phe-glyglygly-L-tyr A.6 (36°C) in the absence and presence of DNA at different DNA base pairs to peptide ratios.



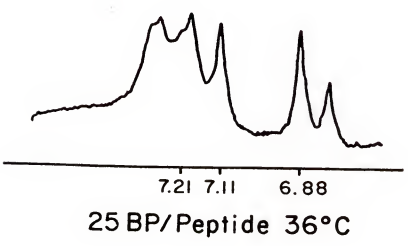


Fig 2-7. Shape of aromatic region for oligopeptide 5 (L-lys-L-phe-glyglygly-L-tyr A) at 8.5 and 25 DNA base pairs to peptide ratios.

Table 2-1. Difference in chemical shifts (in Hz) for the aromatic protons of phenylalanine and tyrosine in the presence and absence of native and denatured DNA.

Aromatic residue of oligopeptides	Δδ (Hz) Base pair/peptide ratios		
	8.5	25	25 (den. DNA)
1 phe	2.6	2.6	17.0
tyr (H 2,6)	1.7	1.7	18.8
tyr (H 3,5)	1.7	1.7	18.2
2 phe	2.4	3.2	19.5
tyr (H 2,6)	0.8	1.0	15.9
tyr (H 3,5)	1.0	1.2	15.7
3 phe*	2.2	3.5	17.1
	3.1	7.4	21.7
average	2.7	5.4	19.4
4 phe	2.8	2.8	11.9
tyr (H 2,6)	2.9	2.9	21.9
tyr (H 3,5)	2.4	2.7	18.8
5 phe**	5.1	5.4	13.2
	9.3	12.9	18.2
average	7.2	9.2	15.7
tyr (H 2,6)	0.7	0.5	16.3
tyr (H 3,5)	0.7	0.7	
6 phe	1.5	2.6	17.0
tyr (H 2,6)	1.4	1.4	12.6
tyr (H 3,5)	1.0	1.0	11.7
L-lys-L-tyr A			
tyr (H 2,6)		9.1	19.4
tyr (H 3,5)		8.2	20.7
L-lys-D-phe A			
phe		2.7	18.07

*Two phenylalanine residues are present. Upon binding of the peptide to DNA two very broad resonances are observed which are upfield shifted on the average by 5.4 Hz at a ratio of 25 base pairs/peptide.

**Two broad resonances are observed for the phenylalanine ring upon binding to DNA which are upfield shifted on the average by 9.2 Hz at a ratio of 25 base pairs to peptide.

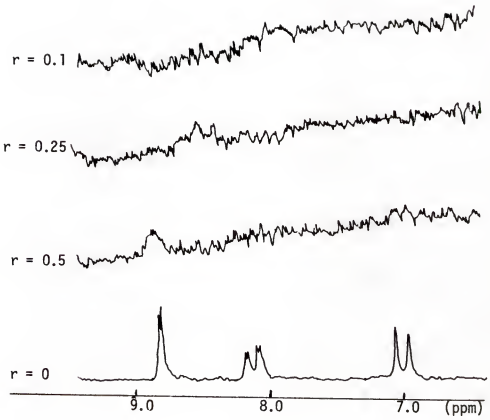


Fig 2-8. ^{1}H NMR signal of the aromatic protons of L-lys-ε-DNP-L-lys A, 9 (36°C) at different molar ratios of DNA-P/reporter (r).

and of peptide 3 (L-lys-L-phe-L-ala-D-phe A). Peptide 3 contains two phenylalanine residues of different chiralities and since the ^{1}H NMR signal of the aromatic protons overlaps, a conclusive assignment as to which phenyl ring is upfield shifted is not possible.

Extensive broadening and large upfield chemical shifts were observed for the aromatic residues of all oligopeptide amides studied in the presence of heat denatured DNA at 36°C. Nonaromatic residues (alanyl and glycyl) were broadened but were not upfield shifted upon binding of the peptides to heat denatured DNA. It should be noted that the line broadening decreases at higher temperatures as the DNA melts out, freeing bound peptide.

The effect of the viscosity of the DNA solution on the line broadening was found to be small as monitored by the sharpness of the internal standard 2,2,3,5-d-4-3-trimethylsilylpropionate (TSP), (width at 1/2 height was found to be around 0.005 ppm and width near base ranged from 0.007-0.017 ppm).

A very large excess of sonicated DNA relative to the oligopeptide amides was used in these experiments (up to a ratio of 25 base pairs/peptide). Gabbay et al.[56] showed that under the conditions of the ^{1}H NMR experiments (7.5 and 15 base pair to peptide ratios) the peptides he studied were almost fully bound to DNA (>95%).

The ^{1}H NMR of peptides 7-9, upon binding to double helical sonicated DNA, showed complete line broadening of the aromatic signals. Figure 2-8 shows the effect of DNA on the ^{1}H NMR signal of the aromatic protons of dipeptide 9 (L-lys-ε-DNP-L-lys A). ^{1}H NMR data could not be obtained for peptides 10 and 11 due to the high insolubility of the DNA-bis-dinitrophenyl peptide complex.

Viscometric Studies

The effective length and, hence, changes in this effective length upon binding a ligand can be related to the intrinsic viscosity (η) of a DNA solution[84-87] according to the following equation:

$$\frac{Lr}{Lm} = \left[\frac{[\eta]_r \, f\,(P)_m}{[\eta]_f \, f\,(P)_r}\right]^{1/3} \qquad 2\text{-}1$$

The subscripts m and r refer to the value for uncomplexed and complexed DNA, respectively. L is the contour length of DNA, $[\eta]$ is the intrinsic viscosity (equation 2-2) and f (P) is a function of the axial ratio of the DNA molecule. The functions $f\,(P)_m$ and $f\,(P)_r$ are fairly insensitive to P at high axial ratios and are frequently assumed to cancel.

Intrinsic viscosity is defined by

$$[\eta] = \lim_{C \to 0} \left(\frac{\eta sp}{C}\right) \qquad 2\text{-}2$$

where ηsp is the specific viscosity of a DNA solution and C is the concentration of the DNA solution.

Unfortunately, intrinsic viscosity experiments for ligand-DNA complexes are generally uninformative since at infinite dilution the intrinsic viscosity of the complex approaches that of free DNA. This is especially true for systems in which the ligand has a low binding constant to DNA. Generally, the viscosity studies are carried out at near infinite dilution of DNA and the relative values of the specific viscosity of the ligand-DNA complex to that of the DNA solution are close approximations to the relative values of the intrinsic viscosities of ligand-DNA complexes.

Therefore, the effect of increasing the concentration of the oligopeptide amides on the relative specific viscosity η_{sp}/η_{spo} (where η_{sp} and η_{spo} are the specific viscosities of the DNA solution in the presence and

absence of the peptide respectively) at 2 x 10^{-4} M DNA-phosphate/liter was studied at 34°C using the lower shear Zimm viscometer. Since a low DNA concentration was used, the relative values of η_{sp}/η_{spo} are approximations of the relative values of the intrinsic viscosity of a DNA-oligopeptide complex to free DNA ($[\eta]/[\eta]_o$).

Oligopeptides 1-6 decrease the specific viscosity of a DNA solution (fig 2-9,2-10). For tetrapeptides the order of decreasing viscosity of DNA was found to be peptide 1 > 3 > 2 and for hexapeptides 5 > 4 > 6. Tetrapeptide 2 initially decreased the viscosity of the DNA solution but afterwards was found to increase it.

Dipeptides 7, 8 and 9 all increased the specific viscosity of DNA at low salt concentration (5 mM Na^+) (fig 2-11). At high salt concentration (0.1 M) peptides 8 and 9 increased the specific viscosity of DNA but not the dipeptide 7 (fig 2-12). Bis-dinitrophenyl peptides, 10 and 11 increased the specific viscosity of DNA to a greater extent than the mono-DNP dipeptides (fig 2-11). Unfortunately, viscosity studies could not be done at the high salt concentration due to solubility problems.

DNA Melting Temperature Studies

The melting temperature of Salmon Sperm DNA in the presence and absence of peptide was determined by monitoring the absorption at 260 nm as the temperature of the DNA solution was gradually increased. This results in a double helix-random coil transition known as denaturation which is characterized by an increase in absorption at 260 nm over a narrow range in temperature whose midpoint is defined as the T_m of the DNA solution.

Tables 2-2 and 2-3 list the ΔT_m values obtained for the DNA-peptide complexes. T_m values are difficult to interpret since the helix-coil

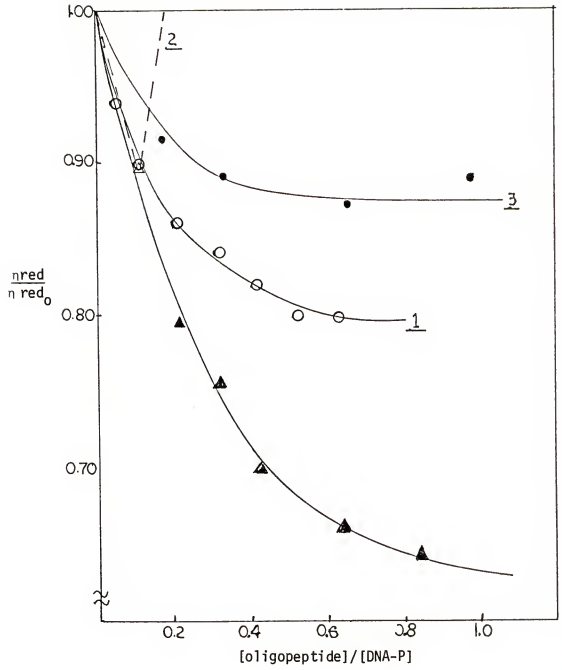


Fig 2-9. Viscometric titration of Salmon Sperm DNA with oligopeptides 1, 2 and 3. Salmon Sperm DNA (1.9 x 10^{-4} M-P/L) was titrated with compounds 1 (O), 2 (Δ---), 3 (●) and L-lys-L-tyr A (▲). The experiments were carried out in 1 x 10^{-2} MES, 1 x 10^{-4} M EDTA, 5 mM Na^+, pH 6.2 at 34°C. $\eta_{red} = \frac{\eta_{sp}}{C_{DNA}}$

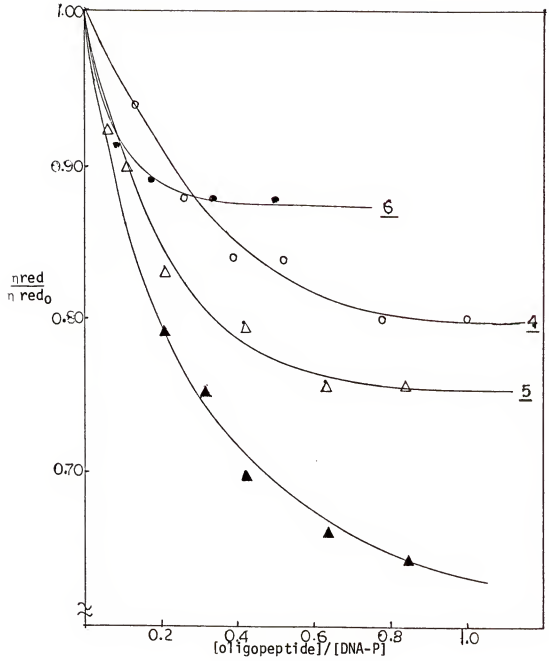


Fig 2-10. Viscometric titration of Salmon Sperm DNA with oligopeptides 4, 5 and 6. Salmon Sperm DNA (1.9×10^{-4} M-P/L) was titrated with compounds 4 (○), 5 (△), 6 (●) and L-lys-L-tyr A (▲). The experiments were carried out in 1×10^{-2} M MES, 1×10^{-4} M EDTA, 5 mM Na^+, pH 6.2 at 34°C.

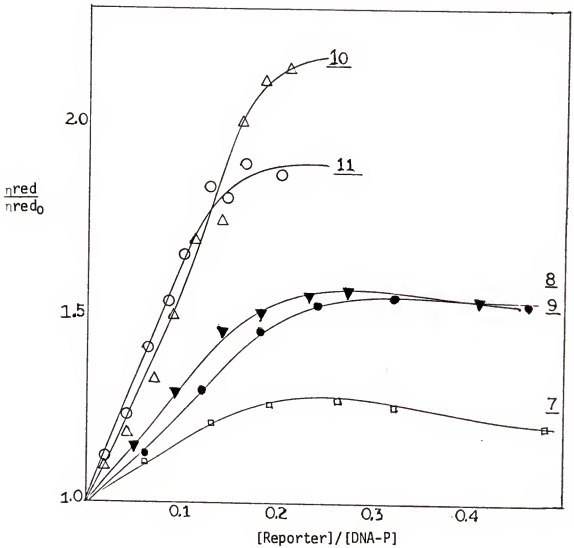


Fig 2-11. Viscometric titration of Salmon Sperm DNA with reporter molecules 7, 8, 9, 10 and 11. Salmon Sperm DNA (2.0×10^{-4} M P/L) was titrated with compounds 7 (□), 8 (▲) 9 (●), 10 (△) and 11 (○). The experiments were carried out in 1×10^{-2} M MES, 1×10^{-4} M EDTA, 5 mM Na^+, pH 6.2 at 34°C.

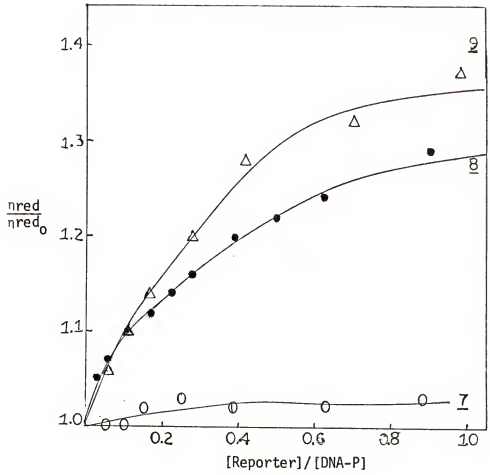


Fig 2-12. Viscometric titration of Salmon Sperm DNA with reporter molecules 7, 8 and 9. Salmon Sperm DNA (2.6×10^{-4} M P/L) was titrated with compounds 7 (O), 8 (●), and 9 (Δ). These experiments were carried out in 1×10^{-2} M MES, 1×10^{-4} M EDTA, 0.1 M NaCl, pH 6.2 at 34°C.

Table 2-2. The effect of oligopeptide amides 1-6 on the helix-coil transition of Salmon Sperm DNA[a]

Oligopeptide	Δ Tm (1.6 pept/DNA-phos)
1 L-lys-L-tyr-L-ala-L-phe A	10.8°C
2 L-lys-D-phe-L-ala-L-tyr A	9.9°C
3 L-lys-L-phe-L-ala-D-phe A	9.8°C
4 L-lys-L-tyr-glyglygly-L-phe A	10.9°C
5 L-lys-L-phe-glyglygly-L-tyr A	9.3°C
6 L-lys-D-phe-glyglygly-L-tyr A	11.3°C
L-lys-L-tyr A*	16.6°C
L-lys-D-Phe A**	10.6°C

[a]The T_m experiments were carried out in 1 x 10^{-2} M MES buffer containing 1 x 10^{-4} M EDTA, 5 mM Na^+, at pH 6.2. The concentration of DNA was 9.4 x 10^{-5} M-P/L at a molar ratio of 1.6 peptide/DNA-P.

*Obtained from Cyclo Chemical Company.

**Prepared by W.D. Wilson.

Table 2-3. The effect of DNP-peptide amides 7-11 on the helix-coil transition of Salmon Sperm DNA.[a]

	DNP-peptide	Δ Tm (0.1 pept/DNA phos)
7	α-DNP-L-lys-L-lys A	15.2°C
8	ε-DNP-L-lys-L-lys A	18.3°C
9	L-lys-ε-DNP-L-lys A	17.2°C
10	L-lys-ε-DNP-L-lys-L-ala-ε-DNP-L-lys A	14.7°C
11	L-lys-ε-DNP-L-lys-$(gly)_3$-ε-DNP-L-lys A	16.6°C

[a]The T_m experiments were carried out in 1×10^{-2} M MES buffer containing 1×10^{-4} M EDTA, 5 mM Na^+ at pH 6.2. The concentration of DNA was 9.4×10^{-5} M-P/L at a molar ratio of 0.1 reporter/DNA-P.

transition involves the interaction of the peptide (P) not only with the helix (H) but also with the coil (C) as shown in the following equation:

$$H + P \overset{k_H}{\rightleftarrows} H.P \rightleftarrows C.P \overset{k_C}{\rightleftarrows} C + P$$

Nevertheless, ΔT_m values can be used to compare the relative binding affinities of the peptides towards DNA.

Circular Dichroism Studies

The effect of increasing concentration of Salmon Sperm DNA on the circular dichroism spectra of compounds 7, 8, 9, 10 and 11 in the range of 300-450 nm was studied. The results shown in figs 2-13, 2-14, 2-15, and summarized in table 2-4, show that a negative induced CD spectra was obtained for all compounds in the region of 300-450 nm. Compounds 7 and 10 exhibit positive CD spectra of their own and upon binding to DNA a negative CD band is induced.

Spectrophotometric Studies

Absorption data for peptides 7, 8, 9, 10 and 11 are summarized in table 2-5. These peptides show an absorption maxima in the vicinity of 360 nm. A typical absorption spectrum of the DNP-monomers and of the bis-DNP peptides is shown in fig 2-16 and 2-17, respectively. A large hypochromic effect was observed for all compounds upon binding to DNA. There was a very small shift of the absorption maxima to longer wavelengths (5-8 nm) for all compounds upon binding to DNA. The percent hypochromicity values are around 80% ± 9% for all molecules. The percent hypochromicity is defined by the following equation $\% H = (1 - \frac{\epsilon_f}{\epsilon_b})\ 100$ where ϵ_f and ϵ_b are the extinction coefficients of the reporter in the absence and presence of DNA under conditions in which the reporter is fully bound to DNA. An isosbestic point was not observed.

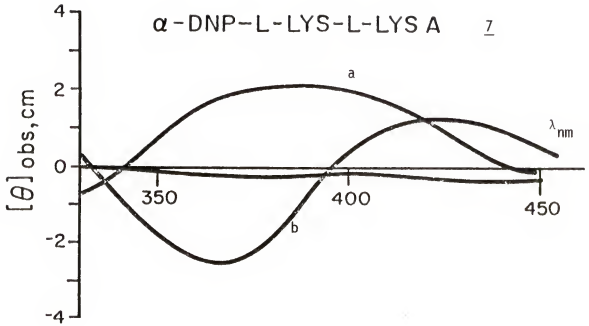


Fig 2-13. The CD spectra of reporter molecule 7 in the absence (a) and presence (b) of Salmon Sperm DNA. The molar ratio of DNA-P to reporter was 20.

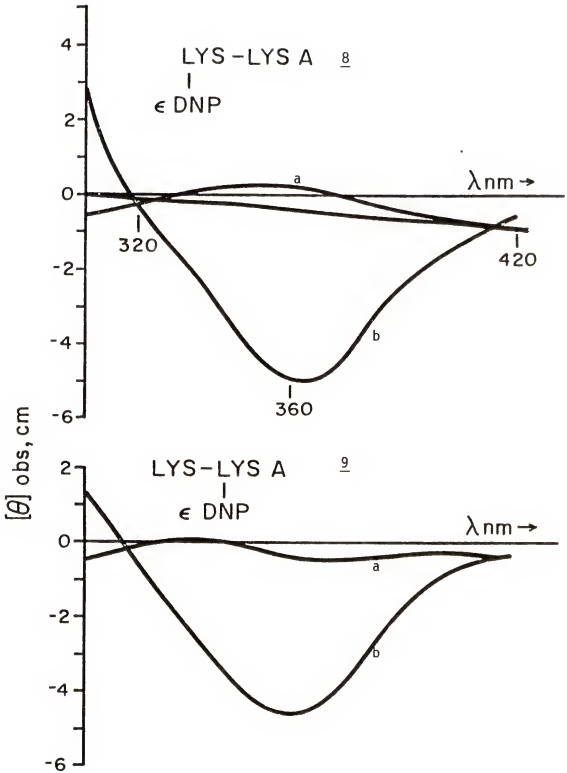


Fig 2-14. The CD spectra of reporter molecules 8 and 9 in the absence (a) and in the presence (b) of DNA. The molar ratio of DNA-P to reporter molecules was 20.

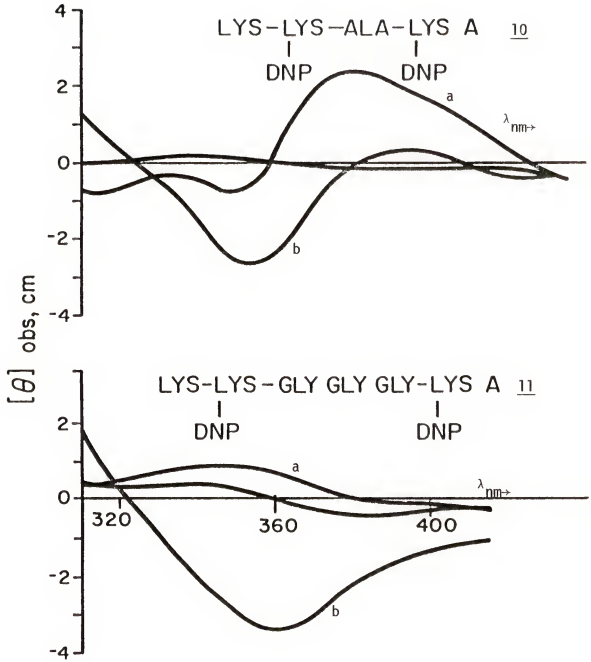


Fig 2-15. The CD spectra of reporter molecules 10 and 11 in the absence (a) and presence (b) of DNA. The molar ratio of DNA-P to reporter molecules was 20.

Table 2-4. Induced circular dichroism data for DNP-peptide amides in the presence of Salmon Sperm DNA.[a,b]

Compound	free λ(nm)	[θ] x 10^{-3}[c]	bound λ(nm)	[θ] x 10^{-3}[c]
7	392	+4.8	*	*
8	——	——	360	-7.3
9	——	——	360	-8.5
10	370	+8.8	*	*
11	——	——	360	-9.8

[a]The CD experiments were carried out in 1 x 10^{-4} M MES buffer containing 1 x 10^{-4} M EDTA, 5 mM Na^{+} at pH 6.2.

[b]Solutions of reporter 7-9 (C ≃ 1 x 10^{-5}) and of reporters 10 and 11 (C ≃ 6 x 10^{-6} M) were titrated with a concentrated solution of Salmon Sperm DNA up to a molar ratio of 0.05 reporter/DNA-P.

[c]The units for [θ] molar ellipticity are dg·cm^2/decimole. $[\theta] = \frac{M_W \cdot \psi}{(\ell \cdot C \cdot 100}$ where M_W is the molecular weight of reporter, ψ is the molecular ellipticity in degrees, ℓ is the cell length in dm, and C is the concentration of reporter in g/mL.

*Unreported due to overlap of CD signals of the bound and unbound reporter to DNA.

Table 2-5. Spectrophotometric data of DNP-peptides 7-11 in the presence and absence of Salmon Sperm DNA.

Compound	free λmax(nm)	E_f[a]	bound λmax(nm)	E_b[b]	% H[c]
7	352	17,600	360	9,300	89
8	360	17,400	365	9.800	78
9	360	16,700	365	9,100	84
10	360	27,100	365	15,700	72
11	360	26,000	365	14,500	79

[a] Extinction coefficient of the reporter in the absence of DNA at 360 nm. The experiments were carried out in 1 x 10^{-2} MES buffer containing 1 x 10^{-4} M EDTA, 5 mM Na^+, pH 6.2 at 25°C.

[b] Extinction coefficient of the bound reporters at 360 nm. The experiments were carried out in the same buffer as above and under conditions in which the reporter peptides were fully bound to DNA ($r \leq 0.01$; r = [Reporter/[DNA-P]).

[c] Percent hypochromicity is defined by $\% H = \left(\frac{\epsilon_f}{\epsilon_b} - 1\right) \times 100$.

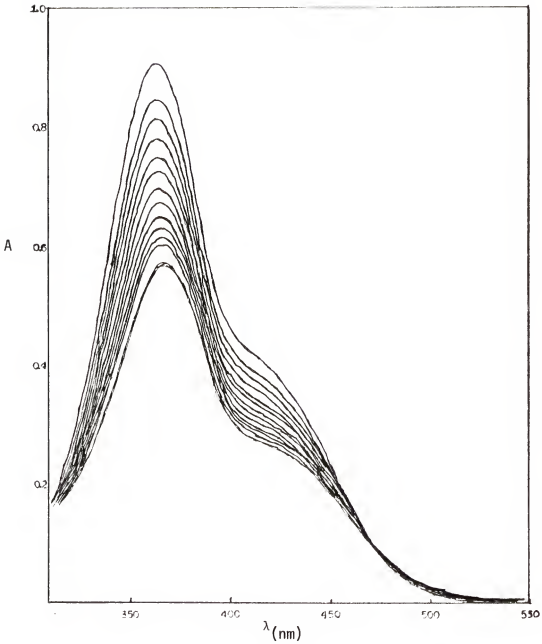


Fig 2-16. The effect of DNA on the absorption spectrum of reporter molecule 8. The spectrum having the largest absorbance at 360 nm is that of free reporter solutions. The molar ratios of DNA-P to reporter were varied from 0-14.

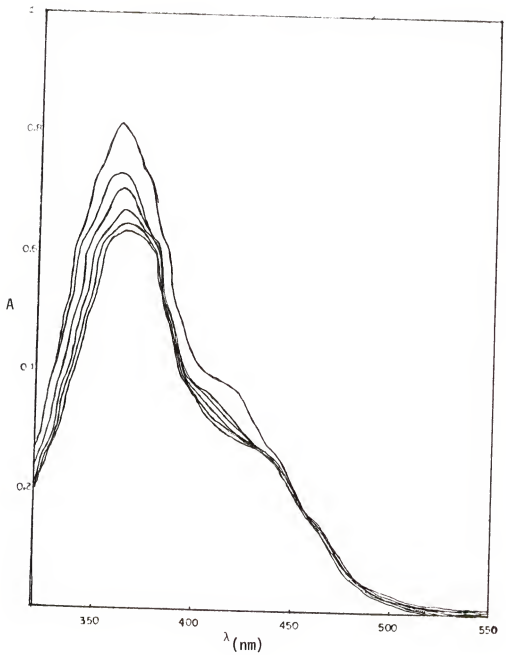


Fig 2-17. The effect of DNA on the absorption spectrum of reporter molecule 11. The spectrum having the largest absorbance at 360 nm is that of free reporter solutions. The molar ratios of DNA-P to reporter were varied from 0-14.

Attempts at determining the relative binding affinities from spectrophotometric titration experiments for reporter molecules 7-11 were unsuccessful, probably due to the complexity of their interaction to DNA (the reporter molecules binding to DNA in more than one manner) as well as due to the low binding affinity of the bis-reporter peptides to DNA.

CHAPTER 3

DISCUSSION

Intercalation of aromatic amino acid residues in between base pairs of DNA is still controversial. Evidence against full insertion of the aromatic moieties has been obtained by several investigators.[62,66] The criteria used has been mostly to compare the results obtained with amino acids and model systems to those obtained with well-known intercalators like ethidium bromide, the acridines, etc.

By studying the tripeptide L-lys-L-trp-L-lys, Dimicoli and Helene[57] concluded that tryptophyl residues are able to intercalate in between base pairs of DNA, but that these will preferentially bind by stacking interactions to single stranded or denatured DNA. Studying a similar tripeptide, L-lys-L-tyr-L-lys, Helene and coworkers[58-61] concluded that tyrosyl residues were unable to intercalate in between base pairs of DNA and that tyrosyl residues could only be capable of stacking interactions with single stranded or denatured DNA.

Gabbay and coworkers[49] took a different approach by designing model systems in which the aromatic portion could insert in between base pairs of DNA to various extents (fig 1-7). They proposed that partial intercalation of the aromatic moiety could occur for some sterically restricted molecules, and that this phenomenon could be distinguished from full intercalation experimentally (decrease in the DNA viscosity vs increase and broadening with upfield shifts of the aromatic protons of the ligand vs total line broadening).

Then Gabbay et al.[56] systemically studied the diastereomeric peptides L-lys-L-phe A and L-lys-D-phe A observing a stereospecific interaction of these with DNA (fig 1-10). They concluded that in one diastereomer (L-lys-L-phe A) the aromatic moiety was pointing into the helix and thus partially intercalated whereas in the other diastereomer it was pointing away from the helix. They also showed that the extent of insertion of phenylalanine into DNA base pairs depended on the position of the phenylalanyl residue in the peptide sequence and on its chirality.

Disagreement in the literature as to whether aromatic amino acids interact with DNA by stacking interactions could be due to 1) choice of peptide studied; 2) comparison of stacking interactions of small ring systems with DNA to those of larger ring systems with DNA with the assumption that stacking interactions of ligands with DNA can occur via a classical intercalative mechanism.

Several types of experimental data can be obtained to determine the extent of insertion of an aromatic moiety in between base pairs of DNA. Viscosity changes have been related to the extent of intercalation.[54] Full intercalation has been characterized by an increase in the viscosity of the DNA solution while a decrease in the viscosity of the DNA solution has been postulated for partial intercalation. It should be noted that electrostatic binding (without intercalation) of cationic molecules to DNA also decreases the viscosity of the DNA solution[52] due to lowering of the electrostatic repulsion existent between phosphate anions in the DNA and thus resulting in shortening of the helix. However, the viscosity decrease in this instance is not as great as observed in those cases where partial intercalation is postulated.

A decrease in the viscosity of a DNA solution upon binding of a ligand capable of stacking interactions cannot be taken alone as evidence for a

partial intercalative mechanism. Other experimental techniques such as ^1H NMR, etc. should be used as added evidence for or against a partial intercalative mechanism.[56]

From ^1H NMR studies, the extent of line broadening and upfield shift can be related to the extent of insertion of an aromatic moiety in between base pairs of DNA. Full intercalation results in total line broadening of the aromatic proton signal. Changes in chemical shifts can be related to the proximity of the aromatic protons of the ligand to the ring current of the DNA base pairs. The ^1H NMR line broadening can be explained by several mechanisms: 1) slow rate of exchange between various DNA binding sites; 2) restricted tumbling of the aromatic rings; 3) differences in the chemical shifts experienced by the various aromatic protons; or 4) a combination of all three mechanisms.

Gabbay and coworkers[54] found that for the dipeptides L-lys-L-phe A and L-lys-D-phe A, under conditions in which the peptides were fully bound to DNA, the T_1 of the aromatic proton peaks for the two diastereomers was of the same magnitude. He concluded that the phenylalanine ring of L-lys-L-phe A was in a different chemical environment in the DNA complex from that of its diastereomer, and that the broadening was a result of the large differences in the chemical shift experienced by the ortho, meta and para protons.

Results from the interaction of peptides 1-6 showed that bis-partial intercalation of the aromatic residues of these peptides was not observed. From ^1H NMR data in table 2-1 it is seen that only the phenylalanine residue of peptide 5 exhibits significant stacking interaction with DNA bases. Two broad resonances were observed for the phenylalanine residue upfield shifted by 5.4 and 12.9 Hz respectively at a ratio of 25 BP/peptide. Comparison of peptides 4, 5 and 6 shows that stacking interactions of

phenylalanine residues with DNA base pairs depend on their position in the sequence (5 vs 4) and on its chirality (5 vs 6). Tyrosine residues for all peptides failed to exhibit stacking interactions and no sequence dependence was observed.

Viscosity results showed that for the hexapeptide series, peptide 5 decreased the viscosity of the DNA solution to a greater extent than peptides 4 and 6. In all cases peptides consisting of all L amino acid residues decreased the specific viscosity of DNA to a greater extent than peptides containing D amino acid residues. The abnormal behavior of peptide 2 on the viscosity of DNA probably results from a conformational change induced on the DNA molecule which results in a dramatic sudden increase in the DNA viscosity. The reason for this nonideal behavior is unknown at this time.

Inspection of the T_m values shown in table 2-2 indicates that all oligopeptide amides stabilize the DNA double helix towards denaturation to a similar degree with the hexapeptide, L-lys-D-phe-glyglygly-L-tyr-A stabilizing it the most.

T_m values represent a measure of a combination of all possible binding interactions of a ligand with DNA. T_m values are affected by electrostatic, hydrophobic interactions as well as by conformational changes occurring upon binding of a ligand to DNA and can be indicative of the strength of binding of the ligand to DNA.

As stated before, no bis-partial intercalation of the studied peptides with DNA base pairs was observed. The inability of these peptides to bis-partially intercalate (peptides 1, 2, 4 and 5) could be related to the inability of the tyrosine residues to engage in stacking interactions with DNA. It should be pointed out that studies on the dipeptide

L-lys-L-tyr A (table 2-1) indicated the presence of stacking interactions upon binding to DNA. A decrease in the viscosity of the DNA was observed as well as broadening and upfield shift of its aromatic protons upon binding to DNA. Furthermore, it has been determined that this dipeptide L-lys-L-tyr A binds more strongly to DNA than L-lys-L-phe A[52,53] and than L-lys-L-tyr-L-lys.[58] Recent studies on the tripeptide L-lys-L-tyr-L-lys and its methylated analog L-lys-O-CH_3-L-tyr-L-lys failed to show a significant difference between the two upon binding to DNA indicating that H-bonding of the tyrosine residue to DNA was not taking place.[61] Also, stacking interactions involving tyrosine residues seem to be dependent on the nature of the neighboring amino acids in the peptide. Upfield shift on the order of 9-10 Hz was observed for L-lys-L-tyr A but very small upfield shift (2-3 Hz) was observed for L-lys-L-tyr-L-lys upon binding to DNA.[58] It should be pointed out that L-lys-L-tyr-L-lys has a free carboxyl group as well as an additional amino group when compared to L-lys-L-tyr A and this might account for the differences observed for the interactions of these two peptides with DNA. Absence of bis-partial intercalation could also be due to the inability of phenylalanine residues not next to L-lysine to engage in stacking interactions.

Surprisingly, results indicate that double stranded DNA may distinguish between phenylalanine and tyrosine residues by stacking preferentially phenylalanine residues in between base pairs of DNA. Upon denaturation of DNA this form of recognition is lost as well as the stereospecificity observed with L-lys-L-phe amide and L-lys-D-phe amide. Table 2-1 shows that extensive broadening and considerable upfield shifts are obtained for all the aromatic residues of the peptide studied as well as for L-lys-L-tyr amide and L-lys-D-phe amide upon binding to heat denatured DNA at 36°C.

Dipeptides 7, 8 and 9 are found to intercalate in between base pairs of DNA at low ionic strength (5 mM Na^+) as seen by viscosity, CD and ^1H NMR studies. The relative specific viscosity of DNA is markedly increased (much more for peptides 8 and 9 than for 7), a negative induced CD is observed and total line broadening of the aromatic proton signals result upon binding to DNA. At higher ionic strength (0.1 M NaCl) dipeptides 8 and 9 still increase the viscosity of DNA but dipeptide 7 has no effect on it. Peptide 7 seems to be interacting with DNA by a nonclassical intercalation mechanism of the type explained before. It binds by intercalation but probably causes some bending at the point of insertion which can result in an overall reduction of the effective length of DNA as seen by a lower viscosity increase when compared to dipeptides 8 and 9.

Bis-DNP-peptides 10 and 11 increase the viscosity of DNA to a much higher extent (almost twice the increase) than dipeptides 8 and 9, which can be considered to be suggestive of bis-intercalation of the two dinitrophenyl moieties. Further evidence is obtained from spectrophotometric studies (table 2-5) which show considerable hypochromism and a small red shift of all DNP peptides upon binding to DNA. The percent hypochromicity values at low salt (5 mM Na^+) are similar for the dipeptides and the bis-DNP-peptides indicating that both DNP rings of the bis-compounds are involved in stacking interactions with DNA. A negative induced CD was obtained for all DNP peptides upon binding to DNA which indicates that the DNP chromophore is in a similar environment in all cases, the asymmetric stacking of base pairs in DNA. According to present theories,[88] the optical activity arises from the interaction of the transition moment(s) of the ligand with the asymmetrically distributed transition moments of the DNA base pairs. It should be pointed out that peptides 7, 10 and to a lesser extent 11 all exhibit a positive CD signal of their own. For peptide 7

this can be explained to be due to the proximity of the chromophore to the asymmetric α carbon of the lysine residue. For the bis-DNP compounds it could be due to the two DNP chromophores interacting asymmetrically with each other, much more in compound 10 due to the proximity of the DNP residues with each other as compared to compound 11. This asymmetric interaction of the two DNP rings with each other is not surprising since the peptide backbone itself provides an asymmetric environment.

As shown in table 2-3, ΔTm values indicate that the peptides 7-11 all stabilize double helical DNA. These ΔTm values are indicative of the relative strength of the binding of these compounds to DNA, and the results show that bis-DNP compounds stabilize DNA to a lesser extent than the mono-DNP compounds. Bis-intercalators studied so far have been found to bind much more strongly than monomeric species to DNA; this is due in part to the increased electrostatic interactions usually present in the dimeric species as compared to the monomer.[76] The mono-DNP and bis-DNP compounds studied here can establish two electrostatic interactions with DNA, between the positively charged ammonium groups of the lysine residues and phosphate groups in the DNA.

Assuming that electrostatic interactions alone are the major stabilizing factor of the DNA-DNP-peptide complex, mono-DNP compounds should show a higher binding affinity for DNA as compared to the bis-DNP compounds. Bis-DNP compounds probably cover more binding sites in the DNA molecules, therefore blocking potential binding sites and in this way decreasing the number of molecules that can interact (either electrostatically or by intercalation) with DNA. Nevertheless, ΔTm values for the bis-DNP compounds are not significantly different from those obtained with the mono-DNP compounds indicating that this loss in electrostatic

interactions is compensated in some way by the increase in hydrophobic interactions (i.e. bis-intercalation).

Conclusion

In this study, attention has been centered around the interaction of peptides with DNA as model systems for the interactions of proteins with DNA. Special attention has been given to the interactions of the aromatic amino acid residues phenylalanine and tyrosine with native and heat denatured DNA. An apparent difference in the way these two residues interact with DNA has been observed and it is suggested that this could be a way by which DNA can distinguish between the two.

Partial intercalation of aromatic residues in between base pairs of DNA is still controversial and it is found to depend on the amino acid (phenylalanine vs. tyrosine), on the position of the aromatic amino acid in the sequence and on the chirality of the aromatic amino acid.

Bis-DNP peptides gave data suggestive of bis-intercalation. Weak binding of these to DNA, when compared to the monomers, is thought to be due primarily to a reduction in the number of electrostatic interactions present in the dimer-DNA complex relative to the monomer-DNA complex. This reduction of the number of electrostatic interactions present is probably caused by the bis-DNP peptide covering more binding sites of the DNA molecule, therefore reducing the number of molecules that can bind to DNA.

CHAPTER 4

MATERIALS AND METHODS

All starting amino acids were purchased from either Sigma Chemical Co. or Vega Biochemicals, and were used without further purification. Salmon Sperm DNA (εp = 6500 at 258 nm)[89] was purchased from Worthington Biochemical Corp. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Georgia. Melting points were taken on a Mel-Temp apparatus and the measurements are uncorrected.

All solutions containing DNA and oligopeptide systems were prepared in buffers made with distilled water. MES [2-(N-morpholino)ethane sulfonic acid] buffer consisted of 1×10^{-2} M MES, 1×10^{-4} M EDTA [ethylenediaminotetra acetic acid], 5 mM Na^{+} at pH 6.2. Phosphate buffers contained 2×10^{-2} M phosphate, 2×10^{-4} M EDTA, pH 6.5.

Synthesis

All the final products and the required intermediates were analyzed for purity by two or more of the following: TLC, NMR, or elemental analysis. All coupling reactions are known to proceed without racemization under the experimental conditions used. The various abbreviations used in this section are 1) THF (tetrahydrofuran); 2) CBZ (carbobenzoxy); 3) BOC (t-butyloxycarbonyl); 4) TFA (trifluoroacetic acid; and 5) DNP (dinitrophenyl). The synthetic scheme shown in figure 4-1 is representative of a typical coupling reaction.

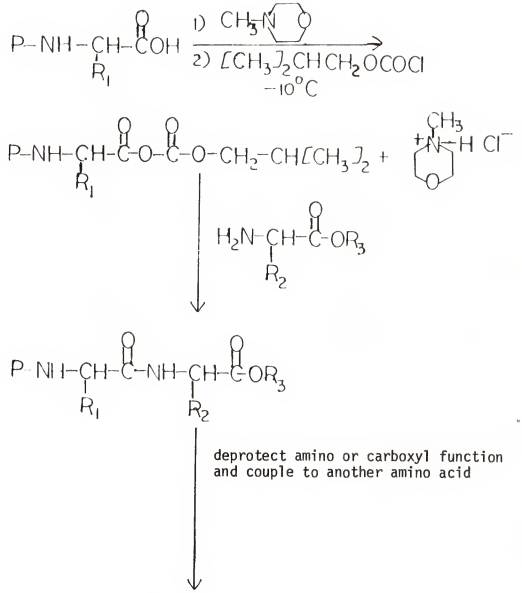


Fig 4-1. Schematic representation of a typical coupling reaction. P (protecting group CBZ or BOC); R_1 R_2 (amino acid residues); R_3 ($-CH_3$ or $-CH_2-CH_3$).

Preparation of L-lysyl-L-tyrosyl-L-alanyl-L-phenylalanine Amide Dihydroacetate, 1.

BOC-L-phenylalanine (4.3 g, 16 mmol) was coupled to aminodiphenylmethane (14 mL, 81.2 mmol) by the mixed anhydride method.[90,91] BOC-L-phenylalanine was dissolved in freshly distilled THF in a three neck flask equipped with a magnetic stirrer and a calcium sulfate drying tube. A stream of dry nitrogen was passed over the solution to keep a moisture free atmosphere. The flask was cooled to -10°C with an acetone/ice bath and 16 mmol of N-methyl morpholine was added to the solution followed by 16 mmol of isobutyl chloroformate. After stirring the mixture for 5 minutes aminodiphenylamine was added and the reaction allowed to stir at the same temperature for 2 hours. Upon completion of the reaction, the solvent was evaporated and the residue dissolved in ethyl acetate/water. The organic phase was washed twice with 0.5 M citric acid, once with water, twice with a saturated $NaHCO_3$ solution and once more with water. The organic phase was then dried over $MgSO_4$, the solvent evaporated, and the residue recrystallized from methanol to yield 4.4 g (10.2 mmol, 64% yield, m.p. 151°C-152°C) of BOC-L-phenylalanyl-(N-diphenylmethyl) amide. The ^1H NMR showed a 9H singlet at δ 1.4 ppm, a 2H doublet at δ 3.1 ppm, a 1H doublet of doublets at δ 4.4 ppm, a 1H at δ 6.2 ppm, a 15H multiplet centered at δ 7.2 ppm, and two broad -NH- resonances at δ 5.0 and 6.6 ppm. Anal. calculated for $C_{27}H_{30}N_2O_3$; C, 75.32; H, 7.02.
Found: C, 75.48; H, 7.10.

BOC-L-phenylalanine-(N-diphenylmethyl) amide was then treated with 50% TFA/CH_2Cl_2 in order to cleave the BOC group. The protected peptide or amino acid is dissolved in 50% TFA/CH_2Cl_2 and the solution stirred for 30 minutes at room temperature. After evaporation of the solvent the residue (the trifluoroacetate salt) was converted to the free amine by

treatment with a saturated $NaHCO_3$ solution and extracted into ethyl acetate. L-phenylalanine-(N-diphenylmethyl) amide (10 mmol) was then coupled to BOC-L-alanine (2.84 g, 15 mmol) by the mixed anhydride method using 15 mmol of N-methyl morpholine and 14 mmol of isobutyl chloroformate. Workup and recrystallization from methanol yielded 4.4 g, (8.8 mmol, 87% yield, m.p. 173°C-176°C) of BOC-L-alanyl-L-phenylalanine-(N-diphenylmethyl) amide. The ^1H NMR in $CDCl_3$ showed a 3H doublet at δ 1.1 ppm, a 9H singlet at δ 1.4 ppm, a 2H multiplet at δ 3.0 ppm, a 1H doublet at 6.2 ppm, and a 15H multiplet at δ 7.2 ppm. Other resonances are observed, mainly -NH- groups, but they are either extensively broadened or their peaks are underneath other peaks.

The BOC group of the dipeptide amide was then cleaved with 50% TFA/CH_2Cl_2 and the residue converted to the free amine as described before, and 1 mmol of it coupled to BOC-O-benzyl-L-tyrosine (1.5 mmol, 557 mg) by the mixed anhydride method using 1.5 mmol of N-methyl morpholine and 1.5 mmol of isobutyl chloroformate. Workup and recrystallization from methanol yielded 450 mg (0.88 mmol, 59% yield) of BOC-O-benzyl-L-tyrosyl-L-alanyl-L-phenylalanine-(N-diphenylmethyl) amide. The ^1H NMR in d_6DMSO showed a 3H doublet at δ 1.2 ppm, a 9H singlet at δ 1.3 ppm, a 2H singlet at δ 5.0 ppm, a 1H doublet at δ 6.1 ppm, a 4H AA'XX' pattern centered at δ 7.00 ppm, and a 15H multiplet at δ 7.2 ppm.

After BOC group cleavage and conversion to the free amine, O-benzyl-L-tyrosyl-L-alanyl-L-phenylalanine-(N-diphenylmethyl) amide (300 mg, 0.46 mmol) was coupled to di-BOC-L-lysine (0.69 mmol) by the mixed anhydride method using 0.69 mmol of N-methyl morpholine and 0.69 mmol of isobutyl chloroformate. Workup and recrystallization from methanol yielded 400 mg (0.40 mmol, 86% yield) of di-BOC-L-lysyl-O-benzyl-L-tyrosyl-L-alanyl-L-phenylalanine-(N-diphenylmethyl) amide. The ^1H NMR in d_6DMSO showed a

18H singlet at δ 1.4 ppm, a broad 6H multiplet at δ 1.6 ppm, a 3H doublet at δ 1.2 ppm, a 2H singlet at δ 5.0 ppm, a 1H doublet at δ 6.1 ppm, a 4H AA'XX' pattern at δ 7.0 ppm, a 15H multiplet at δ 7.2 ppm, and a 5H singlet at 7.4 ppm.

All the protecting groups of the tetrapeptide diphenylmethyl amide were cleaved by treatment with liquid HF. Di-BOC-L-lysyl-O-benzyl-L-tyrosyl-L-alanyl-L-phenylalanine-(N-diphenylmethyl) amide (300 mg, 0.31 mmol) was treated with 15 mL of distilled liquid HF in the presence of 1 mL of anisole.[92] The reaction was allowed to stir at room temperature for an hour. The HF was evaporated and the residue dissolved in 0.1 M HCl/ethyl ether. The organic layer was discarded and the aqueous layer washed twice with ethyl ether. The aqueous layer was lypholized. The residue was dissolved in 2 mL 0.01 M NH_4OAc buffer pH 5.4 and passed through a CM-Sepharose (CL-6B) cation exchange column with a 0.01-0.10 M NH_4OAc gradient. The fractions absorbing at 260 nm were collected and lypholized. Excess NH_4OAc was removed by drying the residue in a vacuum over at 60°C for 24 hours. The peptide amide was recrystallized from methanol/ethyl ether and 90 mg (0.14 mmol, 45% yield) were obtained. The ^1H NMR in D_2O showed a broad 6H multiplet centered at δ 1.6 ppm, a 3H doublet at δ 1.2 ppm, a 6H singlet at δ 1.9 ppm, a 4H AA' XX' pattern pattern at δ 7.0 ppm, and a 5H multiplet at 7.3 ppm, which is consistent with L-lysyl-L-tyrosyl-L-alanyl-L-phenylalanine amide dihydroacetate. Anal. calculated for $C_{31}H_{44}O_9N_6$: C, 57.57; H, 7.17; N, 12.99. Found: C, 57.68; H, 7.20; N, 13.08.

Preparation of L-lysyl-L-phenylalanine-L-alanyl-D-phenylalanine Amide Dihydroacetate, 2.

BOC-D-phenylalanine (5.3 g, 20 mmol) was coupled to aminodiphenylmethane (14 mL, 81 mmol) by the mixed anhydride method, using 20 mmol of

N-methyl morpholine and 20 mmol of isobutyl chloroformate as described previously. Workup and recrystallization from methanol yielded 6 g (14 mmol, 70% yield, m.p. 144°C-145°C) of BOC-D-phenylalanine-(N-diphenylmethyl) amide. The ^1H NMR in $CDCl_3$ shows a 9H singlet at δ 1.4 ppm, a 2H multiplet at δ 3.00 ppm, a 1H multiplet at δ 4.4 ppm, a 1H doublet at δ 6.2 ppm, and a 15H multiplet at 7.3 ppm.

The BOC group was cleaved with 50% TFA/CH_2Cl_2 and the trifluoroacetate salt converted to the free amine. D-phenylalanine-(N-diphenylmethyl) amide (2.9 g, 6.8 mmol) was coupled to BOC-L-alanine by first preparing the symmetrical anhydride BOC-L-alanyl)$_2$O. A solution of dicyclohexylcarbodiimide (14 mmol) in CH_2Cl_2 was added to a stirred cold solution of BOC-L-alanine (28 mmol) in the same solvent. The solution was stirred at 0°C for 1 hour and dicyclohexylurea precipitated as the symmetrical anhydride was formed. The solution was filtered, the filtrate added to 6.8 mmol of D-phenylalanyl-(N-diphenylmethyl) amide dissolved in CH_2Cl_2 and the solution stirred at room temperature overnight. Workup included evaporation of the solvent, dissolving the residue in ethyl acetate/water and washing the organic layer with 0.5 M citric acid, water, saturated $NaHCO_3$ solution and once more with water. Recrystallization from ethyl acetate/ethyl ether yielded 2.2 g (4.4 mmol, 65% yield, m.p. 183°C-185°C) of BOC-L-alanyl-D-phenylalanine-(N-diphenylmethyl) amide. The ^1H NMR in $CDCl_3$ showed a 3H doublet at δ 1.1 ppm, a 9H singlet at δ 1.4 ppm, a 2H multiplet at δ 3.0 ppm, a 1H doublet at δ 6.2 ppm, and a 15H multiplet at δ 7.2 ppm. Other resonances are observed, mainly -NH- groups, but they are either extensively broadened or masked other peaks.

After deprotection with 50% TFA/CH_2Cl_2 L-alanyl-D-phenylalanyl-(N-diphenylmethyl) amide (1.2 g, 3 mmol) was coupled to BOC-L-phenylalanine (5 mmol, 1.3 g) by treatment with dicyclohexylcarbodiimide as described

previously. Workup and recrystallization from ethyl acetate yielded 1.8 g (2.8 mmol, 93% yield, m.p. 204-206°C) of BOC-L-phenylalanyl-L-alanyl-D-phenylalanyl-(N-diphenylmethyl) amide. The ^{1}H NMR in d_6DMSO showed a 3H doublet at δ 1.1 ppm, a 9H singlet at δ 1.3 ppm, a 4H multiplet centered at δ 3.0 ppm, a 1H doublet at δ 6.1 ppm, and a 20H multiplet at δ 7.3 ppm.

The protected tetrapeptide was prepared by coupling di-BOC-L-lysine (1.23 mmol) to L-phenylalanyl-L-alanyl-D-phenylalanine-(N-diphenylmethyl) amide (450 mg, 0.82 mmol) by the mixed anhydride method as described previously. Workup and recrystallization from methanol yielded 704 mg (0.61 mmol, 74% yield, m.p. 228-231°C) of di-BOC-L-lysyl-L-phenylalanyl-L-alanyl-D-phenylalanyl-(N-diphenylmethyl) amide. The ^{1}H NMR in d_6DMSO showed a 3H doublet at δ 1.1 ppm, a 18H singlet at δ 1.4 ppm, a broad 6H multiplet at δ 1.6 ppm, a broad 4H multiplet at δ 3.1 ppm, a 1H doublet at δ 6.1 ppm, and a 20H multiplet at δ 7.2 ppm.

The tetrapeptide amide was obtained by cleaving all the protecting groups with liquid HF at room temperature and purified by chromatography on a CM-Sepharose (CL-6b) cation exchanger as previously described. L-lysyl-L-phenylalanyl-L-alanyl-D-phenylalanine-(N-diphenylmethyl) amide dihydroacetate was recrystallized from methanol/ethyl ether and 80 mg (0.13 mmol, 37% yield) were obtained. The ^{1}H NMR in D_2O showed a 3H doublet at δ 1.1 ppm, a broad 6H multiplet at δ 1.6 ppm, a 6H singlet at δ 1.9 ppm, a 4H broad multiplet centered at δ 3.0 ppm, and a 10H multiplet at δ 7.3 ppm.

Anal. calculated for $C_{31}H_{46}N_6O_8$; C, 59.03; H, 7.35; N, 13.32.
Found: C, 58.89; H, 7.41; N, 13.34.

Preparation of L-lysyl-D-phenylalanyl-L-alanyl-L-tyrosine Amide Dihydroformate, 3.

BOC-D-phenylalanyl-L-alanine methyl ester was prepared by coupling BOC-D-phenylalanine (2 g, 7.5 mmol) with L-alanine methyl ester hydrochloride (1.4 g, 10 mmol) in THF/DMF by the mixed anhydride method using 18 mmol of N-methyl morpholine and 7.5 mmol of isobutyl chloroformate. Workup and recrystallization from ethyl ether/petroleum ether yielded 2 g (5.7 mmol, 77% yield, m.p. 86°C-87°C) of BOC-D-phenylalanyl-L-alanine-methyl ester. The ^1H NMR in $CDCl_3$ showed a 3H doublet at δ 1.3 ppm, a 9H singlet at δ 1.4 ppm, a 2H multiplet at δ 3.0 ppm, a 3H singlet at δ 3.7 ppm, and a 5H multiplet at δ 7.3 ppm.

The ester group of the protected dipeptide ester (1 g, 2.8 mmol) was cleaved by treatment with 1.2 meq of NaOH in methanol. The solution was stirred at room temperature for one hour and then the solvent evaporated. The residue was dissolved in water/ethyl ether, the aqueous layer acidified while cold with 1 N HCl and the product extracted into ethyl acetate. BOC-D-phenylalanyl-L-alanine (0.91 g, 2.7 mmol, m.p. 191°C-193°C) was obtained and used without further purification. BOC-D-phenylalanyl-L-alanine (0.80 g, 2.4 mmol) was then coupled to O-benzyl-L-tyrosine amide (675 mg, 2.5 mmol) in THF by the mixed anhydride method using 2.4 mmol of isobutyl chloroformate and 2.4 mmol of N-methyl morpholine. Workup and recrystallization from ethyl acetate yielded 1.2 g (2 mmol, 86% yield, m.p. 189°C-192°C) of BOC-D-phenylalanyl-L-alanyl-O-benzyl-L-tyrosine amide. The ^1H NMR in d_6DMSO showed a 3H doublet at δ 1.1 ppm, a 9H singlet at δ 1.3 ppm, a 4H multiplet at δ 3.0 ppm, a 2H singlet at δ 5.0 ppm, a 4H AA'XX' pattern at δ 7.0 ppm, a 5H multiplet at δ 7.2 ppm, and a 5H multiplet at δ 7.4 ppm.

The BOC group was cleaved with 35% HBr/acetic acid and the residue converted to the free amine by treatment with $NaHCO_3$. Then D-phenylalanyl-alanyl-O-benzyl-L-tyrosine amide (250 mg, 0.5 mmol) was coupled to diCBZ-L-lysine (414.5 mg, 1 mmol) in THF/DMF by the mixed anhydride method using 1 mmol of isobutyl chloroformate and 1.6 mmol of N-methyl morpholine. Workup and recrystallization from methanol yielded 300 mg (0.34 mmol, 68% yield) of diCBZ-L-lysyl-D-phenylalanyl-L-alanyl-O-benzyl-L-tyrosine amide. The ^1H NMR in d_6DMSO showed a 3H doublet at δ 1.1 ppm, a 4H multiplet at δ 3.0 ppm, a 6H broad singlet at δ 5.0 ppm, a 4H AA' XX' pattern at δ 7.0 ppm, and a 20H multiplet centered at δ 7.3 ppm.
Anal. calculated for $C_{50}H_{56}O_9N_6$; C, 67.86; H, 6.38.
Found: C, 67.84; H, 6.41.

The protecting groups were cleaved by catalytic transfer hydrogenation using formic acid as the hydrogen donor and the Pierce catalyst (a palladium impregnated polymer).[93] The protected tetrapeptide amide (300 mg, 0.34 mmol) was dissolved in 90.5% formic acid and 1 g of the Pierce catalyst added. The flask was rotated on a rotary evaporator for 30 minutes after which the catalyst is recovered by filtration and the solvent evaporated from the filtrate. The residue is dissolved in water and lypholized. Recrystallization from methanol/ethyl ether yielded 120 mg (0.19 mmol, 56% yield) of L-lysyl-D-phenylalanyl-L-alanyl-L-tyrosine amide dihydroformate. The ^1H NMR in D_2O showed a 3H doublet at δ 1.2 ppm, a broad 6H multiplet at δ 1.6 ppm, a 4H multiplet centered at δ 3.0 ppm, a 4H AA' XX' pattern at δ 7.0 ppm, and a 5H multiplet at δ 7.3 ppm.
Anal. calculated for $C_{29}H_{42}O_9N_6 \cdot 3/2\ H_2O$; C, 53.94; H, 7.02.
Found: C, 53.97; H, 7.04.

Preparation of L-lysyl-L-tyrosyl-glycylglycylglycyl-L-phenylalanine Amide Dihydroformate, 4.

CBZ-glycylglycylglycine was prepared by reacting the amino function of glycylglycylglycine (5 g, 26 mmol) obtained from Sigma Chemicals, with benzyl chloroformate (6 mL, 53 meq) in water at 0°C in the presence of 52 meq of $NaHCO_3$. Benzyl chloroformate was added slowly over a 5 minute period and the mixture stirred at this temperature for 90 minutes. The mixture was extracted with 30 mL portions of ethyl ether to remove unreacted benzyl chloroformate and the aqueous layer acidified with 1 N HCl to pH 3 in order to precipitate the protected peptide out of solution. The solid was collected, dried and CBZ-glycylglycylglycine was obtained in 87% yield (7.3 g, 23 mmol). The ^1H NMR in d_6DMSO showed a broad 6H multiplet at δ 3.7 ppm, a 2H singlet at δ 5.0 ppm, and a 5H singlet at δ 7.4 ppm.

CBZ-glycylglycylglycine (600 mg, 1.9 mmol) was then coupled to L-phenylalanine methyl ester hydrochloride (647 mg, 3 mmol) in DMF by the mixed anhydride method using 5 mmol of N-methyl morpholine and 1.9 mmol of isobutyl chloroformate as described previously. Workup and recrystallization from ethyl acetate yielded 570 mg (1.2 mmol, 62% yield). The ^1H NMR in d_6DMSO showed a 2H broad multiplet at δ 3.0 ppm, a 3H singlet at δ 3.7 ppm, a 6H broad multiplet at δ 3.9 ppm, a 2H singlet at δ 5.0 ppm, and a 10H multiplet at δ 7.3 ppm.

Anal. calculated for $C_{24}H_{28}O_7N_4$; C, 59.50; H, 5.83

Found: C, 59.27; H, 5.86.

The CBZ group was cleaved with 35% HBr/HOAc. CBZ-glycylglycylglycyl-L-phenylalanine methyl ester (400 mg, 0.83 mmol) was placed in a round bottom flask equipped with a magnetic stirrer and a calcium sulfate drying tube. A 50 mL portion of a 35% HBr/acetic acid solution was added

and the mixture stirred at room temperature until all the peptide dissolved and gas evolution stopped. The reaction mixture was poured into 100 mL of ethyl ether and the deprotected peptide ester precipitated. The product was filtered under nitrogen, rinsed with more ethyl ether and dried. Recrystallization from methanol/ethyl ether yielded 300 mg (0.77 mmol, 93% yield) of glycylglycylglycyl-L-phenylalanine methyl ester hydrobromide. The ^1H NMR in D_2O showed a 2H broad multiplet at δ 3.0 ppm, a 3H singlet at δ 3.4 ppm, a 6H broad multiplet at δ 3.8 ppm, and a 5H multiplet at δ 7.3 ppm.

Glycylglycylglycyl-L-phenylalanine methyl ester hydrobromide (300 mg, 0.70 mmol) was coupled to N,α-BOC-O-benzyl-L-tyrosine (742 mg, 2 mmol) in THF/DMF by the mixed anhydride method using 2 mmol of isobutyl chloroformate and 3 mmol of N-methyl morpholine. Workup and recrystallization from methanol yielded 400 mg (0.57 mmol, 81% yield) of N,α-BOC-O-benzyl-L-tyrosyl-glycylglycylglycyl-L-phenylalanine methyl ester. The BOC group was then cleaved with 50% TFA/CH_2Cl_2 and the trifluoroacetate salt of the peptide used without further purification. O-benzyl-L-tyrosyl-glycylglycylglycyl-L-phenylalanine methyl ester hydrotrifluoroacetate (300 mg, 0.42 mmol) was coupled to di-CBZ-L-lysine (829 mg, 2 mmol) in THF/DMF by the mixed anhydride method using 2 mmol of isobutyl chloroformate and 3 mmol of N-methyl morpholine. Workup and recrystallization from methanol yielded 300 mg (0.30 mmol, 71% yield) of di-CBZ-L-lysyl-O-benzyl-L-tyrosyl-glycylglycylglycyl-L-phenylalanine methyl ester.

Anal. calculated for $C_{54}H_{61}N_7O_{12}$; C, 64.85; H, 6.15.
Found: C, 64.80; H, 6.17.

The hexapeptide ester was deprotected by catalytic transfer hydrogenation using formic acid as the hydrogen donor and freshly prepared palladium black as the catalyst. Palladium black was prepared according

to the procedure given by Greenstein and Winitz[94] but starting with palladium acetate instead of palladium metal. Di-CBZ-L-lysyl-O-benzyl-L-tyrosyl-glycylglycylglycyl-L-phenylalanine methyl ester (300 mg, 0.3 mmol) was dissolved in 90.5% formic acid and 300 mg of freshly prepared palladium black added. The reaction mixture was stirred at room temperature under nitrogen for 2 hours after which the solvent was evaporated under reduced pressure and the residue dissolved in water, lypholized and dried. The ^1H NMR in D_2O showed a 6H broad multiplet at δ 1.6 ppm, a 3H singlet at δ 3.4 ppm, a 6H broad multiplet at δ 3.8 ppm, a 4H AA'XX' pattern at δ 7.0 ppm, and a 5H multiplet at δ 7.3 ppm, which is consistent with L-lysyl-L-tyrosyl-glycylglycylglycyl-L-phenylalanine methyl ester dihydroformate.

The hexapeptide ester was converted to the amide by treatment with a methanol solution saturated with ammonia gas. The hexapeptide ester (0.3 mmol) was dissolved in methanol cooled in an ice/acetone bath and ammonia gas bubbled through the solution until saturation. A stream of nitrogen was passed over the reaction mixture to keep a moisture free atmosphere. After saturation the bottle was securely capped and the reaction mixture left for 2 days at room temperature. After 2 days the bottle was cooled in an ice/acetone bath, opened and the solvent evaporated. The residue was recrystallized from methanol/ethyl ether to yield 70 mg (0.10 mmol, 33% yield) of L-lysyl-L-tyrosyl-glycylglycylglycyl-L-phenylalanine amide dihydroformate. The ^1H NMR in D_2O showed a broad 6H multiplet at δ 1.6 ppm, a 4H multiplet at δ 3.1 ppm, three 2H singlets centered at δ 3.9 ppm, a 4H AA'XX' pattern centered at δ 7.0 ppm, and a 5H multiplet at δ 7.3 ppm.

Anal. calculated for $C_{32}H_{46}O_{11}N_8$; C, 53.47; H, 6.45; N, 15.59.
Found: C, 53.35; H, 6.49; N, 15.49.

Preparation of L-lysyl-L-phenylalanyl-glycylglycylglycyl-L-tyrosyl Amide Dihydroformate, 5.

Di-CBZ-L-lysyl-L-phenylalanine (1.8 mmol, 1 g) was coupled to glycylglycylglycine ethyl ester hydrochloride (3 mmol, 760 mg) in DMF/THF by the mixed anhydride method using 5.4 mmol of N-methyl morpholine and 1.8 mmol of isobutyl chloroformate. Workup and recrystallization from ethanol yielded 0.96 g (1.3 mmol, 69% yield) of di-CBZ-L-lysyl-L-phenylalanyl-glycylglycylglycine ethyl ester. The ^{1}H NMR in d_6DMSO showed a broad 6H multiplet at δ 1.6 ppm, a 6H multiplet at δ 3.8 ppm, a 3H triplet at δ 1.2 ppm, a 2H quartet at δ 4.1 ppm, a 2H singlet at δ 5.0 ppm, and a 15H multiplet at δ 7.3 ppm.

The ester (0.48 g, 0.63 mmol) was cleaved with 1.0 meq of NaOH/methanol. The reaction mixture was warmed over a steam bath until it became homogeneous and then stirred at room temperature for 30 minutes, after which the solvent was evaporated. The residue was dried *in vacuo* over P_2O_5 and used without further purification. Di-CBZ-L-lysyl-L-phenylalanyl-glycylglycylglycine (0.4 g, 0.55 mmol) was coupled to O-benzyl-L-tyrosine amide by the mixed anhydride method using 0.55 mmol of isobutyl chloroformate and 0.6 mmol of N-methyl morpholine as described previously. After evaporation of the solvent a saturated $NaHCO_3$ solution was added to the residue and the mixture stirred. The solid residue was filtered, washed with water and dried. The solid was added to methanol, filtered (impurities are soluble in methanol but not the protected peptide) and then dried to yield 230 mg (0.23 mmol, 42% yield, m.p. 220°C-222°C) of di-CBZ-L-lysyl-L-phenylalanyl-glycylglycylglycyl-O-benzyl-L-tyrosine amide. The ^{1}H NMR in d_6DMSO showed a broad 6H multiplet at δ 1.6 ppm, a 6H broad multiplet at δ 3.8 ppm, a 6H broad singlet at δ 5.0 ppm, a 4H AA'XX' pattern at δ 7.0 ppm, and a 20H multiplet at δ 7.3 ppm.

The protecting groups were cleaved by catalytic transfer hydrogenation using formic acid as the hydrogen donor and freshly prepared palladium black as the catalyst. Di-CBZ-L-lysyl-L-phenylalanyl-glycylglycylglycyl-O-benzyl-L-tyrosine amide (200 mg, 0.20 mmol) was dissolved in 90.5% formic acid and 200 mg of freshly prepared palladium black added. The reaction mixture was stirred at room temperature under nitrogen for 2 hours after which the solvent was evaporated under reduced pressure and the residue dissolved in water and lypholized. The product was recrystallized from methanol/ethyl ether and 80 mg (0.10 mmol) of L-lysyl-L-phenylalanyl-glycylglycylglycyl-L-tyrosine amide dihydroformate were obtained. The ^1H NMR showed a broad multiplet at δ 1.6 ppm, a 4H multiplet at δ 3.0 ppm, three 2H singlets centered at δ 3.9 ppm, a 4H AA'XX' pattern at δ 7.0 ppm, and a 5H multiplet at δ 7.3 ppm.

Anal. calculated for $C_{32}H_{46}O_{11}N_8 \cdot 1/2\ H_2O$; C, 52.81; H, 6.51; N, 15.40. Found: C, 52.77; H, 6.52; N, 15.36.

Preparation of L-lysyl-D-phenylalanyl-glycylglycylglycyl-L-tyrosine Amide Dihydroformate, 6.

BOC-D-phenylalanine (4.0 g, 15.1 mmol) was coupled to glycylglycylglycine ethyl ester hydrochloride (2.6 g, 10.2 mmol) by the mixed anhydride method as described previously. Workup and recrystallization from ethyl acetate/ethyl ether yielded 2.8 g (6 mmol, 60% yield, m.p. 142°C-144°C) of BOC-D-phenylalanyl-glycylglycylglycine ethyl ester. The ^1H NMR in $CDCl_3$ showed a 9H singlet at δ 1.3 ppm, a 3H triplet at δ 1.2 ppm, a 6H broad multiplet at δ 3.8 ppm, a 2H quartet at δ 4.1 ppm, and a 5H multiplet at δ 7.3 ppm.

The ester group was then cleaved with NaOH in methanol as described previously and the residue used without further purification. BOC-D-phenylalanyl-glycylglycylglycine (200 mg, 0.46 mmol) was then coupled to

O-benzyl-L-tyrosine amide (270 mg, 1 mmol) by the mixed anhydride method. Workup and recrystallization from methanol/ethyl ether yielded 260 mg (0.38 mg, 83% yield, m.p. 170°C-172°C) of BOC-D-phenylalanyl-glycylglycylglycyl-O-benzyl-L-tyrosine amide. The BOC group was deprotected with 50% TFA/CH_2Cl_2 and the pentapeptide amide (320 mg, 0.54 mmol) was coupled to di-CBZ-L-lysine (414.5 mg, 1 mmol) using 1 mmol of isobutyl chloroformate and 1 mmol of N-methyl morpholine. Workup and recrystallization from methanol (impurities are soluble in methanol but not the protected peptide) yielded 280 mg (0.28 mmol, 52% yield) of di-CBZ-L-lysyl-D-phenylalanyl-glycylglycylglycyl-O-benzyl-L-tyrosine amide. The ^1H NMR in d_6DMSO showed a broad 6H multiplet at δ 1.6 ppm, a 6H broad multiplet at δ 3.8 ppm, a broad 6H singlet at δ 5.0 ppm, a 4H AA'XX' pattern at δ 7.0 ppm, and a 20H multiplet at δ 7.3 ppm.

Anal. calculated for $C_{53}H_{60}O_{11}N_8$; C, 64.62; H, 6.14.
Found: C, 64.42; H, 6.20.

The protecting groups were cleaved by catalytic transfer hydrogenation using formic acid as the hydrogen donor and the Pierce catalyst (a palladium impregnated polymer) as the catalyst. The protected hexapeptide amide (273 mg, 0.28 mmol) was dissolved in 90.5% formic acid and 1 g of the Pierce catalyst added. The flask was rotated on a rotary evaporator for 30 minutes after which the catalyst is recovered by filtration and the solvent evaporated from the filtrate. The residue is dissolved in water and lypholized. Recrystallization from methanol/ethyl ether yielded 70 mg (36% yield) of L-lysyl-D-phenylalanyl-glycylglycylglycyl-L-tyrosine amide dihydroformate. The ^1H NMR in D_2O showed a broad 6H multiplet at δ 1.6 ppm, a 4H multiplet at δ 3.0 ppm, three 2H singlets centered at δ 4.0 ppm, a 4H AA'XX' pattern centered at δ 7.0 ppm, and a 5H multiplet at δ 7.3 ppm.

Anal. calculated for $C_{32}H_{46}O_{11}N_8 \cdot 1H_2O$; C, 52.17; H, 6.57; N, 15.21. Found: C, 52.05; H, 6.60; N, 15.08.

Preparation of N,α-DNP-L-lysyl-L-lysine Amide Dihydrobromide, 7.

N,α-BOC-N,ε-CBZ-L-lysine (5 g, 13 mmol) was converted to a mixed anhydride by treatment with isobutyl chloroformate (13 mmol) in the presence of N-methyl morpholine (13 mmol) in THF at -10°C under a nitrogen atmosphere. A stream of ammonia gas was bubbled through the solution at this temperature until saturation and then the solvent evaporated. The residue was dissolved in ethyl acetate/water and the organic phase washed with saturated $NaHCO_3$ and then with water. The organic phase was dried over $MgSO_4$ and the solvent evaporated. Recrystallization from methanol/ethyl ether yielded .7 g (9 mmol, 75% yield, m.p. 134°C-136°C) of N,α-BOC-N,ε-CBZ-L-lysine amide. The BOC group was then cleaved by treatment with 50% TFA/CH_2Cl_2 at room temperature for 30 minutes after which the solvent was evaporated, the residue converted to the free amine by treatment with saturated $NaHCO_3$ and extracted into ethyl acetate.

N,α-DNP-N,ε-CBZ-L-lysine was prepared by first cleaving the BOC group of N,α-BOC-N,ε-CBZ-L-lysine with 50% TFA/CH_2Cl_2 and reacting the resulting trifluoroacetate salt (880 mg, 2.2 mmol) with dinitrofluorobenzene in methanol, in the presence of 4.4 mmol of N-methyl morpholine. The mixture was stirred until it became homogeneous and the solvent then evaporated. The residue was dissolved in ethyl acetate, washed with 0.5 M citric acid, and then with water. The organic phase was dried and the solvent evaporated to yield an orange-yellow oil which was used without further purification.

N,α-DNP-N,ε-CBZ-L-lysine (0.32 g, 0.72 mmol) was then coupled to N,ε-CBZ-L-lysine amide (396 mg, 1.5 mmol) by the mixed anhydride method

using 1.1 mmol of N-methyl morpholine and 0.72 mmol of isobutyl chloroformate. Workup and recrystallization from ethanol/ethyl ether yielded 400 mg N,α-DNP-N,ε-CBZ-L-lysyl-N,ε-CBZ-L-lysine amide (0.58 mmol, 80% yield, m.p. 181°C-183°C). The ^{1}H NMR in $CDCl_3$ showed a broad 12H multiplet at δ 1.4 ppm, a broad 4H multiplet at δ 3.0 ppm, a 4H singlet at δ 5.0 ppm, a 10H singlet at δ 7.3 ppm, a 1H doublet at δ 7.0 ppm, a 1H doublet of doublets at δ 8.3 ppm, and a 1H doublet at δ 9.0 ppm.

The di-CBZ-DNP-dipeptide amide (350 mg, 0.5 mmol) was deprotected by treatment with 35% HBr/acetic acid in a flask fitted with a calcium chloride drying tube. Reaction is complete when solid is dissolved and gas evolution stops. Upon completion of the reaction the deprotected peptide amide was precipitated by an excess of ethyl ether and the solid filtered under nitrogen. Recrystallization from methanol/ethyl ether yielded 250 mg (0.40 mmol, 71% yield) of N,α-DNP-L-lysyl-L-lysine amide dihydrobromide. The ^{1}H NMR in D_2O, 0.02 M phosphate buffer, 0.0002 M EDTA, pH 6.5, showed a broad 12H multiplet at δ 1.6 ppm, a broad 4H multiplet at δ 3.0 ppm, two methine triplets centered at δ 4.5 and 4.3 ppm, a 1H doublet at δ 7.0 ppm, a 1H doublet of doublets at δ 8.3 ppm, and a 1H doublet at δ 9.1 ppm.

Anal. calculated for $C_{18}H_{31}O_6N_7Br_2$; C, 39.95; H, 5.20.
Found: C, 38.86; H, 5.23.

Preparation of N,ε-DNP-L-lysyl-L-lysine Amide Dihydrobromide, 8.

N,α-BOC-L-lysine (4.5 g, 16 mmol) was reacted with dinitrofluorobenzene (2.96 g, 16 mmol) in the presence of 32 mmol of N-methyl morpholine in methanol. The mixture was stirred at room temperature until it became homogeneous and the solvent evaporated. The residue was dissolved in ethyl acetate and washed with 0.5 M citric acid followed by water. The solvent was then evaporated and the resulting yellow oil (5 g) chromatographed

on silica gel and eluted with 50% $CHCl_3/CH_3OH$·A dark yellow oil was obtained (3.3 g, 7.5 mmol, 47% yield). The ^1H NMR in $CDCl_3$ showed a 6H singlet at δ 1.4 ppm, a broad 6H multiplet at δ 1.7 ppm, a 2H multiplet centered at δ 3.4 ppm, a 1H multiplet at δ 4.2 ppm, a 1H doublet at δ 6.9 ppm, a doublet of doublets at δ 8.2 ppm, and a 1H doublet at δ 8.9 ppm, which is consistent with N,α-BOC-N,ε-DNP-L-lysine.

The DNP protected amino acid (282 mg, 0.75 mmol) was then coupled to N,ε-CBZ-L-lysine amide (396 mg, 1.5 mmol) by the mixed anhydride method using 0.75 mmol of N-methyl morpholine and 0.75 mmol of isobutyl chloroformate. Workup and recrystallization from methanol/water yielded 339 mg (0.51 mmol, 68% yield, m.p. 134°C-136°C). The ^1H NMR in $CDCl_3$ showed a 9H singlet at δ 1.4 ppm, a broad 12H multiplet at δ 1.7 ppm, a broad 4H multiplet at δ 3.2 ppm, a broad 4H multiplet at δ 4.2 ppm, a 2H singlet at δ 5.0 ppm, a 5H singlet at δ 7.3 ppm, a 1H doublet at δ 6.9 ppm, a 1H doublet of doublets at δ 8.2 ppm, and a 1H doublet at δ 9.1 ppm, which is consistent with N,α-BOC-N,ε-DNP-L-lysyl-N,ε-CBZ-L-lysine amide.

The protected DNP-dipeptide amide (320 mg, 0.48 mmol) was deprotected with 35% HBr/acetic acid. Workup and recrystallization from methanol/ethyl ether yielded 228 mg (0.38 mmol, 80% yield) of N,ε-DNP-L-lysyl-L-lysine amide dihydrobromide, 8. The ^1H NMR in D_2O, 0.02 M phosphate buffer, 0.0002 M EDTA, pH 6.5, showed a broad 12H multiplet at δ 1.6 ppm, a 2H methylene triplet at δ 3.0 ppm, a 2H methylene triplet at δ 3.5 ppm, a 1H methine triplet at δ 4.0 ppm, a 1H methine triplet at δ 4.3 ppm, a 1H doublet at δ 7.2 ppm, a 1H doublet of doublets at δ 8.3 ppm, and a 1H doublet at δ 9.1 ppm.

Anal. calculated for $C_{18}H_{31}O_6N_7BR_2$; C, 35.95; H, 5.20.
Found: C, 35.97; H, 5.20.

Preparation of L-lysyl-L-N,ε-DNP-lysine Amide Dihydrobromide, 9.

L-N,ε-DNP-lysine hydrochloride was synthesized according to the general method of Sanger.[95] L-lysine mono hydrochloride (20 g, 0.11 mmol) was dissolved in hot water and excess $CuCO_3$ (40.6 g, 0.33 mmol) was added. After removing excess $CuCO_3$ by filtration, a dark blue solution was obtained and the volume reduced to half. $NaHCO_3$ (28 g, 0.33 mol) was added, followed by dinitrofluorobenzene (20.5 g, 0.11 mmol) dissolved in ethanol. The solution was stirred for two hours at room temperature and a green precipitate formed which was filtered and dried. The green solid was then dissolved in 1 M HCl and 9.9 g (0.13 mol) of thioacetamide was added. The solution was heated for one hour on a steam bath to precipitate CuS. Activated charcoal was added and the solution filtered. The filtrate was evaporated and the solid obtained recrystallized from 1 M HCl to yield 23 g (0.06 mmol, 60% yield) of L-N,ε-DNP-lysine hydrochloride. The ^1H NMR in d_6DMSO showed a broad 6H multiplet at δ 1.7 ppm, a broad 2H methylene multiplet at δ 3.6 ppm, a broad 1H methine multiplet at δ 4.0 ppm, a 1H doublet at δ 7.3 ppm, a 1H doublet of doublets at δ 8.2 ppm, and a 1H doublet at δ 8.8 ppm.

Anal. calculated for $C_{12}H_{17}O_6Cl$; C, 41.33; H, 4.91.

Found: C, 41.10; H, 4.98.

L-N,ε-DNP-lysine was then converted to the ester by treatment of 5.3 g (0.015 mmol) with dry ethanol saturated with HCl gas. Workup and recrystallization from ethanol/ethyl ether gave 4.2 g (0.11 mmol, 73% yield, m.p. 156°C-158°C) of L-N,ε-DNP-lysine ethyl ester hydrochloride. The ^1H NMR in D_2O showed a 3H methyl triplet at δ 1.2 ppm, a broad 6H multiplet at δ 1.7 ppm, a broad 2H methylene multiplet at δ 3.3 ppm, a broad 3H multiplet at δ 4.2 ppm, a 1H doublet at δ 6.7 ppm, a 1H doublet of doublets at δ 7.8 ppm, and a 1H doublet at δ 8.3 ppm.

The ester was then coupled to di-BOC-L-lysine by the mixed anhydride procedure. Di-BOC-L-lysine (519 mg, 1.5 mmol) was converted to the mixed anhydride by treatment with 1.4 mmol of isobutyl chloroformate in the presence of 1.5 mmol of N-methyl morpholine in THF at -10°C. A DMF solution containing 1 mmol each of L-N,ε-DNP-lysine ethyl ester hydrochloride and N-methyl morpholine was added and the reaction was allowed to stir at -10°C for 2 hours. Upon completion of the reaction, the solvent was evaporated and the residue dissolved in ethyl acetate/water. The organic phase was washed with 0.5 M citric acid, water, saturated $NaHCO_3$, and once more with water. The organic phase was then dried over $MgSO_4$ and the solvent evaporated to yield di-BOC-L-lysyl-L-N,ε-DNP-lysine ethyl ester (oil obtained, ca. 1 mmol yellow oil). The protected dipeptide ester was the converted to the amide by saturating a methanol solution containing the dipeptide ester with ammonia gas at -10°C in a high pressure bottle. The bottle was securely capped and allowed to stand at room temperature for 48 hours after which the bottle was opened and the solvent evaporated. The residue was recrystallized from methanol/ethyl ether to yield 470 mg (0.74 mmol, 74% yield). The ^1H NMR in $CDCl_3$ showed a 18H singlet at δ 1.4 ppm, a broad 12H multiplet at δ 1.7 ppm, a broad 4H methylene multiplet at δ 3.2 ppm, a broad multiplet centered at δ 4.2 ppm, a 1H doublet at δ 6.9 ppm, a 1H doublet of doublets at δ 8.2 ppm, and a 1H doublet at δ 9.1 ppm.

Di-BOC-L-lysyl-L-N,ε-DNP-lysine amide (400 mg, 0.62 mmol) was deprotected by treatment with 35% HBr in acetic acid. Workup and recrystallization from methanol/ethyl ether yielded 320 mg (0.53 mmol, 85% yield) of L-lysyl-N,ε-DNP-L-lysine amide dihydrobromide. The ^1H NMR in D_2O, 0.02 M phosphate buffer, 0.0002 M EDTA, pH 6.5, showed a broad 12H multiplet at δ 1.6 ppm, a 2H methylene triplet at δ 3.0 ppm, a 2H methylene

triplet at δ 3.5 ppm, a 1H methine triplet at δ 4.0 ppm, a 1H methine triplet at δ 4.3 ppm, a 1H doublet at δ 7.2 ppm, a 1H doublet of doublets at δ 8.3 ppm, and a 1H doublet at δ 9.1 ppm.

Anal. calculated for $C_{18}H_{31}O_6N_7Br_2$; C, 35.95; H, 5.20.

Found: C, 35.80; H, 5.20.

Preparation of L-lysyl-N,ε-DNP-L-lysyl-L-alanyl-N,ε-L-lysine Amide Dihydroacetate, 10.

N,α-BOC-N,ε-DNP-L-lysine (2 g, 5.3 mmol) was coupled to L-alanyl methyl ester hydrochloride (1.1 g, 8 mmol) by the mixed anhydride method using 14 mmol of N-methyl morpholine and 5.3 mmol of isobutyl chloroformate. After usual workup of 2 g (4.3 mmol, 81% yield) an orange oil was obtained. The methyl ester was cleaved by dissolving 2 g of it in methanol and adding 1.2 meq of 2 N NaOH and the solution stirred for an hour at room temperature. After evaporation of the solvent the residue was treated with 1.2 meq of 1 N HCl and extracted into ethyl acetate. An oil was obtained after evaporation of the solvent which was chromatographed on silica gel and eluted with 25% methanol/$CHCl_3$ to yield 1.4 g (2.9 mmol of a dark yellow oil). The ^1H NMR showed a 6H singlet at δ 1.4 ppm, a broad 9H multiplet at δ 1.5 ppm, a doublet at δ 6.9 ppm, a doublet of doublets at δ 8.1 ppm, and a doublet at δ 8.9 ppm.

The protected DNP-dipeptide (1.4 g, 2.9 mmol) was then coupled to N,ε-DNP-L-lysine ester hydrochloride (1.1 g, 2.9 mmol) by the mixed anhydride method, using 2.9 mmol of isobutyl chloroformate and 5.8 mmol of N-methyl morpholine. After usual workup the oil residue was chromatographed on silica gel and eluted with 25% methanol/$CHCl_3$ to yield 1 g (1.2 mmol) of N,α-BOC-N,ε-DNP-L-lysyl-L-alanyl-N,ε-DNP-L-lysine ethyl ester, an oil. The ^1H NMR in $CDCl_3$ showed a 6H singlet at δ 1.4 ppm, a 3H doublet at δ 1.2 ppm, a broad 12H multiplet at δ 1.7 ppm, a broad 4H

multiplet at δ 3.4 ppm, a 2H quartet at δ 4.2 ppm, a 3H broad multiplet at δ 4.4 ppm, a 2H doublet at δ 6.8 ppm, a 2H doublet of doublets at δ 7.9 ppm, and a 2H doublet at δ 8.8 ppm.

The protected di-DNP-tripeptide ester (1 g, 1.2 mmol) was then converted to the amide by treatment with a methanol solution saturated with ammonia gas as described before. After workup and recrystallization from ethyl acetate/ethyl ester 756 mg (0.97 mmol, 81% yield) of N,α-BOC-N,ε-DNP-L-lysyl-L-alanyl-N,ε-DNP-L-lysine amide were obtained. The ^{1}H NMR in d_6DMSO showed a 6H singlet at δ 1.4 ppm, a 3H doublet at δ 1.3 ppm, a broad 12H multiplet at δ 1.6 ppm, a 4H broad multiplet at δ 3.5 ppm, a broad 3H multiplet at δ 4.1 ppm, a 2H doublet at δ 7.2 ppm, a 2H doublet of doublets at δ 8.3 ppm, and a doublet at δ 8.8 ppm.
Anal. calculated for $C_{32}H_{44}O_{13}N_{10}$; C, 49.48; H, 5.71.
Found: C, 49.56; H, 5.73.

The BOC group of the di-DNP-tripeptide amide was cleaved with 50% TFA/CH_2Cl_2 as described previously and the residue converted to the free amine by treatment with $NaHCO_3$. N,ε-DNP-L-lysyl-L-alanyl-N,ε-DNP-L-lysine amide (330 mg, 0.5 mmol) was then coupled to di-CBZ-L-lysine by the mixed anhydride method using 1.2 mmol of N-methyl morpholine and 1 mmol of isobutyl chloroformate. After workup, the resulting solid was found to be sparingly soluble in methanol (hot or cold), but impurities were quite soluble in methahol. The residue was added to methanol, heated on a steam bath, filtered while hot and dried to yield 300 mg (0.28 mmol, 56% yield). The ^{1}H NMR in d_6DMSO showed a broad 18H multiplet centered at δ 1.7 ppm, a broad 6H multiplet centered at δ 3.4 ppm, a broad 4H multiplet centered at δ 4.2 ppm, a 4H singlet at δ 5.0 ppm, a 10H singlet at δ 7.3 ppm, a 2H doublet at δ 7.2 ppm, a 2H doublet of doublets at δ 8.3 ppm, and a 2H doublet at δ 8.8 ppm.

Anal. calculated for $C_{49}H_{60}O_{16}N_{12}$; C, 54.85; H, 5.64.
Found: C, 54.71; H, 5.70.

The CBZ's groups of the di-DNP-tetrapeptide amide were cleaved with 35% HBr/acetic acid as described previously. The resulting solid was contaminated with an inorganic impurity as determined by elemental analysis, and was chromatographed on a CM-Sepharose (CL-6B) cation exchange column. The fraction eluted with 0.5 M NH_4OAc (pH 5.4) was collected, lypholized, dried and 50 mg (0.05 mol) of L-lysyl-N,ε-DNP-L-lysyl-L-alanyl-N,ε-L-lysine amide dihydroacetate were obtained. The ^1H NMR in D_2O, 0.01 M MES buffer, 0.0001 M EDTA, pH 6.2, at 70°C showed a broad 27H multiplet centered at δ 1.8 ppm, a broad doublet at δ 6.9 ppm, a broad doublet at δ 8.0 ppm, and a broad singlet at δ 8.7 ppm. At 90°C the peaks were sharper and more defined, the alanine methyl doublet at δ 1.2 ppm can be distinguished from the lysine's methylenes, in the aromatic region a sharp doublet at δ 7.0 ppm, a doublet of doublets at δ 8.1 ppm, and a broad singlet at δ 8.8 ppm were observed.
Anal. calculated for $C_{37}H_{56}O_{16}N_{12}\cdot 3/2\ H_2O$; C, 46.68; H, 6.25.
Found: C, 46.64; H, 6.26.

Preparation of L-lysyl-N,ε-DNP-L-lysyl-(glycyl)$_3$-N,ε-DNP-L-lysine Amide Dihydrobromide, 11.

Di-CBZ-L-lysyl-N,ε-DNP-L-lysine ethyl ester was prepared as previously described for the synthesis of the di-BOC derivative. The di-CBZ-DNP-dipeptide ethyl ester was obtained in 78% yield. The ethyl ester was cleaved by treating the protected dipeptide ester (1 g, 1.4 mmol) with 1.2 meq of 2 N NaOH in methanol. Di-CBZ-L-lysyl-N,ε-DNP-L-lysine (oil) was obtained in 68% yield (0.82 g, 1.2 mmol).

CBZ-glycylglycylglycine was prepared by reacting the amino function of glycylglycylglycine (5 g, 26 mmol), obtained from Sigma Chemicals, with

benzylchloroformate (6 mL, 53 meq) in water at 0°C in the presence of 52 meq of $NaHCO_3$. Benzyl chloroformate was added slowly over a 5 minute period and the mixture stirred at this temperature for 90 minutes. The mixture was extracted with three 30 mL portions of ethyl ether to remove unreacted benzyl chloroformate and the aqueous layer acidified with 1 N HCl to pH 3 in order to precipitate the protected peptide out of solution. The solid was collected, dried and CBZ-glycylglycylglycine was obtained in 87% yield (7.3 g, 23 mmol). The ^1H NMR in d_6DMSO showed a broad 6H multiplet at δ 3.7 ppm, a 2H singlet at δ 5.0 ppm, and a 5H singlet at δ 7.4 ppm.

CBZ-glycylglycylglycine (646 mg, 2 mmol) was then coupled to N,ε-DNP-L-lysine ethyl ester hydrochloride (350 mg, 0.93 mmol) by the mixed anhydride method using 2.4 mmol of N-methyl morpholine and 2 mmol of isobutyl chloroformate. Workup and recrystallization from ethyl acetate/ethyl ester yielded 480 mg (0.74 mmol, 80% yield) of CBZ-glycylglycyl-glycyl-N,ε-DNP-L-lysine ethyl ester. The ^1H NMR in d_6DMSO showed a 3H triplet at δ 1.2 ppm, a broad 6H multiplet at δ 1.4 ppm, a broad 6H triplet (3 singlets) centered at δ 3.8 ppm, a 2H quartet at δ 4.1 ppm, a 2H singlet at δ 5.1 ppm, a 5H singlet at δ 7.4 ppm, a 1H doublet at δ 7.3 ppm, a 1H doublet of doublets at δ 8.3 ppm, and a 1H doublet at δ 8.9 ppm.

The CBZ group was then cleaved by treating 400 mg (0.62 mmol) of the CBZ-DNP-tetrapeptide ester with 35% HBr/acetic acid. Workup and recrystallization from ethanol/ethyl ether yielded 270 mg (0.46 mmol, 74% yield) of glycylglycylglycyl-N,ε-DNP-L-lysyl ethyl ester hydrobromide.

Di-CBZ-L-lysyl-N,ε-DNP-L-lysine (315 mg, 0.44 mmol) was then coupled to glycylglycylglycyl-N,ε-DNP-L-lysine ethyl ester (592 mg, 0.42 mmol) by the mixed anhydride method using 1 mmol of isobutyl chloroformate. Workup,

which included filtering the residue from methanol, yielded 360 mg (0.30 mmol) of di-CBZ-L-lysyl-N,ε-DNP-L-lysyl-glycylglycylglycyl-N,ε-DNP-L-lysine ethyl ester. The ^{1}H NMR in d_6DMSO showed a 3H triplet at δ 1.3 ppm, a broad 18H multiplet at δ 1.6 ppm, a broad 6H multiplet at δ 3.8 ppm, a 2H quartet at δ 4.2 ppm, a 4H singlet at δ 5.1 ppm, a broad 2H doublet at δ 7.3 ppm, a 10H singlet at δ 7.4 ppm, a broad 4H multiplet at δ 8.2 ppm, and a 2H doublet at δ 8.9 ppm.

The CBZ groups of the di-DNP-hexapeptide ester (200 mg, 0.17 mmol) were cleaved with 35% HBr/acetic acid and the residue converted to the amide by treatment with a solution of methanol saturated with ammonia gas. L-lysyl-N,ε-DNP-L-lysyl-glycylglycylglycyl-N,ε-DNP-L-lysine amide dihydrobromide was recrystallized from methanol/ethyl ether and 50 mg (0.05 mmol, 30% yield) were obtained. The ^{1}H NMR in D_2O, 0.01 M MES buffer, 0.0001 M EDTA, pH 6.2, at 36°C showed a broad 18H multiplet at δ 1.7 ppm, a broad 6H multiplet at δ 3.97 ppm, a 2H doublet at δ 7.0 ppm, a 2H doublet of doublets at δ 8.2 ppm, and a 2H doublet at δ 8.8 ppm. Anal. calculated for $C_{36}H_{54}O_{14}N_{14}Br_2$; C, 40.53; H, 5.10. Found: C, 40.74; H, 5.20.

^{1}H NMR Studies

The ^{1}H NMR spectra were recorded with either a Jeol-JNM-FX 100 Fourier transform or a Varian A-60A NMR spectrometer. For ^{1}H NMR studies involving the interactions of peptides with DNA, sonicated Salmon Sperm DNA was used. Sonicated Salmon Sperm DNA was dissolved in phosphate buffer containing 2×10^{-2} M phosphate, 2×10^{-4} M EDTA, 4 mM Na^+ at pH 6.5. This solution was lypholized, dissolved in D_2O, lypholized again and redissolved in D_2O to give a solution that was 0.26 M DNA-P. Peptide solutions in phosphate buffer were lypholized, dissolved in D_2O, lypholized again and redissolved in D_2O.

The ^1H NMR of the peptides and of DNA-peptide complexes (at two different base pair to peptide ratios) were taken on a Jeol-FX 100 spectrometer at 36°C. At the highest base pair to peptide ratio, the nmr spectra were also measured at 90°C after which the nmr tube was cooled to 36°C and the nmr spectra taken again (heat denatured DNA). Chemical shifts (in Hz) are reported relative to the internal standard TSP (sodium 3-trimethylsilylpropionate-2,2,3,3-d_4).

Viscometric Studies

Viscosity measurements were performed with a low shear Zimm viscometer (Beckman Instrument Co.). For the viscosity measurements, the viscometer was thermostated at 34°C with a Haake constant-temperature circulator. The specific viscosity (η_{sp}) of a DNA solution was calculated by relating the time required for one rotation of the rotor inside the viscometer for a DNA solution (τ_{DNA}) relative to that of the buffer (τ_{buffer}) according to the following equation:

$$\eta_{sp} = \frac{\tau_{DNA} - \tau_{buffer}}{\tau_{buffer}}$$

A peptide solution was titrated into the DNA solution in the viscometer and the effect of the peptides on the specific viscosity of DNA was determined. MES buffer 1 x 10^{-2} M, 1 x 10^{-4} M EDTA, 5 mM Na^+, pH 6.2 was used.

Absorption Studies

Absorption spectra (550 nm-320 nm) of the DNP peptides were recorded on a Cary-17D uv/vis spectrophotometer in a 1 cm cell at 25°C using MES buffer. A solution of the peptide (A ≈ 0.9) was placed in the cell and the spectra recorded and the peptide titrated with several DNA aliquots and the spectra recorded again. The extinction coefficient for the reporter bound to DNA (ε_b) was determined by titrating a reporter solution into a concentrated DNA solution and reading the absorbance at 360 nm.

A plot of absorbance vs reporter concentration resulted in a straight line whose slope gave the bound reporter extinction coefficient. The same procedure was followed to obtain the extinction coefficient of the free species (ε_f), but this time titrating the reporter molecule into buffer. Linear regression analysis of the data yielded correlation coefficients of 0.999 or better for both cases. Percent hypochromicity values were calculated as follows:

$$\% H = \left(\frac{\varepsilon_f}{\varepsilon_b} - 1\right) 100$$

Circular Dichroism Studies

A Jasco J-20 spectropolarimeter was used to record circular dichroism measurements. CD spectra of reporter and bis-reporter peptides were taken in a 5 cm cell in the region of 300 nm-500 nm at ambient temperature using MES buffer. These reporter solutions were titrated with a concentrated DNA solution up to a reporter/DNA-P ratio of 0.05 after which titration with DNA did not affect the observed CD spectra anymore.

Melting Temperature (T_m) Studies

T_m measurements were run in 1 mL quartz cuvettes thermostated with a Haake constant-temperature circulator equipped with a constant speed motor to provide a heating rate of 1°/minute. A Gilford model 240 spectrometer equipped with automatic recording accessories was used and the temperature of the cell compartment was measured directly with an iron-constantan thermocouple connected to a chart recorder which had been standardized at 0°C and 100°C.

A 9.4×10^{-5} M solution of Salmon Sperm DNA (1 mL) was placed in each of the cells and peptides 1-6 were added in a sufficient amount to give a ratio of 1.5 pept/DNA-P. Reporter peptides 7-11 were added in sufficient amount to give a ratio of 0.1 pept/DNA-P.

BIBLIOGRAPHY

1. Gabbay, E. J. and Wilson, W. D. Methods in Cell Biology, vol. 18, p. 351 (1978), Academic Press, New York.

2. Blake, A. and Peacock, A.R. Biopolymers, 6, 1225 (1968).

3. Cavalieri, E. and Calvin, M. Proc. Natl. Acad. Sci. USA, 68, 1251 (1971).

4. Muller, W. and Crothers, D. M. J. Mol. Biol., 35, 251 (1968).

5. Wang, J. C. J. Mol. Biol., 89, 983 (1974).

6. Pillai, C. K. S. and Nandi, U. S. Biopolymers, 12, 1431 (1973).

7. Shapiro, J. T., Stannard, B. S. and Felsenfeld, G. Biochemistry, 8, 3233 (1969).

8. Felsenfeld, G. Nature, 271, 115 (1978).

9. Kornberg, A. Science, 163, 1410 (1969).

10. Jacob, F. and Monod, J. J. Mol. Biol., 3, 318 (1961).

11. Bram, S. J. Mol. Biol., 58, 277 (1971).

12. Bram, S. and Beeman, W. W. J. Mol. Biol., 55, 311 (1971).

13. Watson, J. D. and Crick, F. H. C. Nature, 171, 737 (1953).

14. Watson, J. D. and Crick, F. H. C. Nature, 171, 964 (1953).

15. Arnott, S. Prog. Biophys. Mol. Biol., 21, 267 (1970).

16. Langridge, R., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F. and Hamilton, L. P. J. Mol. Biol., 2, 19 (1960).

17. Andrew, H., Wang, J., Quigley, G. J., Kolpak, F. J., Crawford, S. L., van Boom, J. H., van der Marel, G. and Rich, A. Nature, 282, 689 (1979).

18. Crick, F. H. C. and Klug, A. Nature, 255, 530 (1975).

19. Sobell, H. M., Tsai, C., Gilbert, S. G., Jain, S. C. and Sakore, T. D. Proc. Natl. Acad. Sci. USA, 73, 3068 (1976).

20. Olson, W. K. Biopolymers, 18, 1213 (1979).

21. Olson, W. K. Biopolymers, 18, 1235 (1979).

22. Kyogoku, Y., Lord, R. C. and Rich, A. Biochim. Biophys. Acta, 179, 10 (1969).

23. Leng, M. and Felsenfeld, G. J. Mol. Biol., 15, 455 (1966).

24. Crothers, D. M. and Ratner, D. I. Biochemistry, 7, 1823 (1968).

25. Olins, D. E. J. Mol. Biol., 43, 439 (1969).

26. Gabbay, E. J. and Glasser, R. Biochemistry, 10, 1665 (1971).

27. Lerman, L. S. J. Mol. Biol., 3, 18 (1961).

28. Lerman, L. S. Proc. Natl. Acad. Sci. USA, 49, 94 (1963).

29. Lerman, L. S. J. Cell Comp. Physiol., 64, 1 (1964).

30. Cohen, G. and Eisenberg, H. K. Biopolymers, 8, 45 (1969).

31. Saucier, J. M., Festy, B. and LePecq, J. B. Biochimie, 53, 973 (1971).

32. Waring, M. J. Mol. Biol., 54, 247 (1970).

33. Vinograd, J., Revet, B. M. J. and Schmir, M. Nature New Biology, 229, 10 (1971).

34. Pulleyblank, D. E. and Morgan, A. R. J. Mol. Biol., 91, 1 (1975).

35. Neville, D. M. and Davies, D. R. J. Mol. Biol., 17, 57 (1966).

36. Lippard, S. J., Jennette, K. W., Vassiliades, G. A. and Bauer, W. R. Proc. Natl. Acad. Sci. USA, 71, 3839 (1974).

37. Crothers, D. M. Biopolymers, 6, 575 (1968).

38. Sobell, H. M., Sakore, T. D. and Reddy, B. S. J. Mol. Biol., 135, 763 (1979).

39. Sobell, H. M., Sakore, T. D., Reddy, B. S. and Seshadri, T. P. J. Mol. Biol., 135, 787 (1979).

40. Sobell, H. M., Jain, S. C., Bhandary, K. K. J. Mol. Biol., 135, 813 (1979).

41. Wang, A. H. J., Quigley, G. J. and Rich, A. Nuc. Acids Res., 6, 3879 (1979).

42. Neidle, S., Dabrow, M., Berman, H. M. and Shieh, H. S. Nuc. Acids Res., 8, 85 (1980).

43. Rhodes, W. J. Am. Chem. Soc., 83, 3609 (1961).

44. Ellerton, N. F. and Isenber, I. Biopolymers, 8, 767 (1969).

45. LePecq, J. B. and Paoletti, C. J. Mol. Biol., 27, 87 (1967).

46. Lee, C. H., Chang, G. J. and Wetmur, J. Biopolymers, 12, 1099 (1973).

47. Blears, D. J. and Danyluk, S. S. Biopolymers, 5, 535 (1967).

48. Gabbay, E. J., Gaffney, B. L. and Wilson, L. A. Biochem. Biophys. Res. Comm., 35, 854 (1969).

49. Kapicak, L. and Gabbay, E.J. J. Am. Chem. Soc., 97, 403 (1975).

50. Gabbay, E. J., Scofield, R. E. and Baxter, C. S. J. Am. Chem. Soc., 95, 7850 (1973).

51. Brown, P. E. Biochim. Biophys. Acta, 213, 282 (1970).

52. Gabbay, E. J., Sanford, K. and Baxter, S. C. Biochemistry, 11, 3429 (1972).

53. Gabbay, E. J., Sanford, K., Baxter, S. C. and Kapicak, L. Biochemistry, 12, 4021 (1973).

54. Adawakar, P., Wilson, W. D., Brey, W. and Gabbay, E. J. J. Am. Chem. Soc., 97, 1959 (1975).

55. Gabbay, E. J., Adawakar, P. D. and Wilson, W. D. Biochemistry, 15, 146 (1976).

56. Gabbay, E. J., Adawakar, P. D., Kapicak, L., Pearce, S. and Wilson, W. D. Biochemistry, 15, 1521 (1976).

57. Dimicoli, J. L. and Helene, C. Biochemistry, 13, 714 (1974).

58. Dimicoli, J. L. and Helene, C. Biochemistry, 13, 724 (1974).

59. Toulme, J. J. and Helene, C. J. Biol. Chem., 252, 244 (1977).

60. Mayer, R., Toulme, F., Montenay-Garestier, T. and Helene, C. J. Biol. Chem., 254, 75 (1979).

61. Toulme, F., Helene, C., Fuchs, R. P. P. and Daune, M. Biochemistry, 19, 870 (1970).

62. Jacobsen, J. and Wang, J. C. Biochem. Biophys. Acta, 335, 49 (1973).

63. Quadrifoglio, F., Crescenzi, V. and Giaconti, V. Biophys. Chem., 1, 319 (1974).

64. Quadrifoglio, F. and Crescenzi, V. Biophys. Chem., 2, 64 (1974).

65. Quadrifoglio, F., Crescenzi, V., Prota, G., Cariello, L., DiMarco, A. and Zunino, F. Chem. Biol. Interactions, 11, 91 (1975).

66. Quadrifoglio, F., Giaconti, V. and Crescenzi, V. FEBS Letters, 65, 345 (1976).

67. Livshitz, M. A., Gursky, G. V., Zasedatelev, A. S. and Volkenstein, M. V. Nuc. Acids Res., 6, 2217 (1979).

68. Waring, M. J. and Wakelin, L. P. G. Nature, 252, 653 (1974).

69. Ugetto, G. and Waring, M. J. Mol. Pharmacol., 13, 579 (1977).

70. Chung, H. T., Feeney, J., Roberts, G. C. K., Williams, D. H., Ugetto, G. and Waring, M. J. J. Am. Chem. Soc., 100, 46 (1978).

71. Wakelin, L. P. G. and Waring, M. J. Biochem. J., 157, 721 (1976).

72. Shafer, R. H. Biopolymers, 19, 431 (1980).

73. LePecq, J. B., LeBret, M., Barbet, J. and Roques, B. Proc. Natl. Acad. Sci. USA, 72, 2915 (1975).

74. Canellakis, E. S., Shaw, Y. H., Hanners, W. E. and Schwartz, R. A. Biochim. Biophys. Acta, 418, 277 (1976).

75. Chen, T. K., Fico, R. and Canellakis, E.S. J. Med. Chem., 21, 868 (1978).

76. Gaugain, B., Barbet, J., Oberlin, R., Roques, B.P. and LePecq, J. B. Biochemistry, 17, 5071 (1978).

77. Pelaprat, D., Delbarre, A., LeGuer, I., Roques, B.P. and LePecq, J. B. J. Med. Chem., 23, 1336 (1980).

78. Pelaprat, D., Delbarre, A., LeGuer, I., Roques, B.P. and LePecq, J. B. Biochemistry, 19, 5537 (1980).

79. Barbet, J., Roques, B.P., Combrisson, S. and LePecq, J. B. Biochemistry, 15, 2642 (1976).

80. Capelle, N., Barbet, J., Dessen, P., Blanket, S., Roques, B.P. and LePecq, J. B. Biochemistry, 18, 3354 (1979).

81. Wright, R. G. McR., Wakelin, L. P. G., Fields, A., Acheson, R. M. and Waring, M. J. Biochemistry, 19, 5825 (1980).

82. Wakelin, L. P. G., Romanos, M., Chen, T. K., Glaubiger, D., Canellakis, E. S. and Waring, M. J. Biochemistry, 17, 5057 (1978).

83. Gabbay, E. J. J. Am. Chem. Soc., 91, 5136 (1969).

84. Cohen, G. and Eisenberg, H. Biopolymers, 8, 45 (1969).

85. Voordouw, G., Kam, Z., Borochov, N. and Eisenberg, H. Biophys. Chem., 8, 171 (1978).

86. Godfrey, J. E. and Eisenberg, H. Biophys. Chem., 5, 301 (1976).

87. Knowles, P. F., Marsh, D. and Rattle, H. W. E. Magnetic Resonance of Biomolecules, p. 1-70 (1976) John Wiley & Sons, Ltd., New York.

88. Tinoco, I. Jr., Bustamante, C. and Maestre, M. F. Ann. Rev. Biophys. Bioeng., 9, 107 (1980).

89. Chargaff, E., Lipschitz, R., Green, C. and Hodes, M. E. J. Mol. Biol., 192, 223 (1951).

90. Anderson, G. W., Zimmerman, J. E. and Callahan, F. M. J. Am. Chem. Soc., 89, 5012 (1967).

91. Izeboud, E. and Beyerman, H. C. Rec. Trav. Chim., 97, 1 (1978).

92. Hruby, V. J., Muscio, F. A., Brown, W. and Gitu, P. M. Chemistry and Biology of Peptides, p. 331 (1972) Ann Arbor Science Publishers, Michigan.

93. Pierce Chemical Company.
ElAmin, B., Anantharamaiah, G. M., Royer, G. P. and Means, G. E. J. Org. Chem., 44, 3442 (1979).

94. Greenstein, J. P. and Winitz, M. Chemistry of the Amino Acids, vol. 3, p. 1233 (1961) Wiley, New York.

95. Sanger, F. Biochem. J., 39, 507 (1945).

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